

# Cytotoxic and Antioxidant Activities of Selected South African Medicinal Plants

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## ABSTRACT

**Introduction:** Indigenous plants are known to play a pivotal role in the well-being of Africa's native societies. These plants are believed to be endowed with healing effects against various ailments rooting from oxidative stress. However, most of these plants including *Ziziphus mucronata*, *Warburgia salutaris* and *Eucomis autumnalis* lack scientific reports validating their medicinal properties. Thus, the current study, aimed to investigate the cytotoxic and antioxidant activities of selected South African medicinal plants. **Methods:** Scavenging activity of the extracts were evaluated on 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide anion (O<sub>2</sub><sup>-</sup>) nitric oxide (NO<sup>-</sup>), hydroxyl anion (·OH) radicals and lipid peroxide (LP) values at varying concentration (20 - 100 µg/mL). Cytotoxic evaluation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was conducted on HEK293 cells. Reducing power and metal chelating ability of the extracts were assessed. In the same vein, protective index of DNA plasmid (pET151/D TOPO plasmid) induced free radical by incubating with Fenton's reagents were tested, *in vitro*. Reducing and metal chelating ability of the extracts were evaluated in this study. **Results:** The crude extracts displayed radical scavenging activity and limit peroxidation process. The extracts showed potent ferric reducing power and chelating effects. Cytotoxic effects of the extracts analyzed using the MTT assay displayed low toxicity levels. Furthermore, the extracts were able to protect pET151/D-TOPO plasmid DNA nicking induced by hydroxyl radicals in a Fenton's reaction mixture. **Conclusion:** It is evident in this study that the plant extracts are potential antioxidant therapeutics and justify inclusion of indigenous herbs as a complementary agents in the treatment regimen.

**Key Words:** Antioxidants, DNA, *Eucomis autumnalis*, *Warburgia salutaris*.

## INTRODUCTION

Empirical usage of medicinal plants to treat diverse ailments and systematic investigation of their acclaimed properties, constitute the main focus of the current research trend in the main time.<sup>1,2</sup> Over the past decades, a number of medicinal plants have been at the epicenter of drug discovery.<sup>3-5</sup> This is due to their ability to protect sensitive and crucial organs that are prone to oxidative damaged initiated by the complex of reactive radical molecules generated in the biological system.<sup>5,6</sup> The generation of these reactive oxygen species (ROS) is a well-known aerobic process, crucial for maintaining normal cellular functions such as signal transduction.<sup>7</sup> Evaluation of ROS is based on the extent of oxidative challenges to macromolecular blocks; DNA, proteins, lipid and are thought to be major factor responsible for various pathophysiological derangements that are experiencing in ageing and various oxidatively induced diseases.<sup>8-10</sup>

Administration of both synthetic and natural antioxidant molecules have been shown to prevent the advancement of free radicals.<sup>11</sup> Thus, the inhibition of radical species by natural molecular derivative have been a motivation towards progressive and systematic search for agents among native herbs that can inhibit toxic onslaught imposed by free radicals and with therapeutic

ability to repair damaged done to the liver and other tissues. The search for such plants among the native herbs is urgently called for and as such worthy of empirical investigation.<sup>12</sup> Free radical inhibitory effects with potent regenerative properties of good number of medicinal plants have been tested for and there were laudable empirical findings. Significant amounts of these plants' constituents limit and facilitate the regeneration of natural healing process of damaged liver, without eliciting toxicity on the experimental subjects model employed in the course of the investigation.<sup>2,13-17</sup> Plant extract and their derivatives are currently in short supply due to high demand especially on their usage as alternative source of primary health care in developing world, owing to the low cost, availability and safety interms of treatment and management of liver disorders.<sup>18</sup>

*Eucomis autumnalis* (Mill) (*Hyacinthaceae*) Chitt is found distributed around southeastern-southern African Sub-region.<sup>19</sup> The specie has been long time employed locally in the treatment of varieties of ailments and as well as facilitating child birth within the Province of Kwazulu-Natal, South African.<sup>14,20</sup> Antimicrobial and anti-inflammatory activity of the extract fractions was earlier investigated *in vitro*. The findings showed that, the bulbs exhibit moderate anti-microbial and anti-inflammatory property, wound healing facilitator and a better dietary supplements to a victim of autoimmune diseases.<sup>14</sup>

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Eucosterol, 16 $\beta$ -hydroxy-eucosterol and Eucosterol glycoside are the natural nortriterpenes family isolated thus far from *Eucomis* species.<sup>21,22</sup>

*Ziziphus mucronata* is a member of *Rhamnaceae* family, drought tolerant with ability to withstand high temperature. They are known as buffalo thorn and are evenly distributed throughout summer rainfall areas of Sub-Saharan Africa, extending from South Africa northwards to Ethiopia and Arabia. The roots and leaves of *Z. mucronata* have been reportedly used to treat wounds and bacteria infections, while the stem bark are being used to improve and beautify skin appearance.<sup>23</sup> The outcome of the review conducted by Olajuyigbe and Afolayan, (2011), on the various phenolic constituents and antioxidants activity of the stem bark on this plant, showed that several medicinal herbs used as treatment option of various ailments, despite their inbuilt natural pharmacological efficacies that have been exploited in the traditional health care setting, are yet to be adequately subjected to empirical validation.<sup>24</sup> A pentacyclic triterpenes compound known as Betulinic acid has been isolated from this herb.<sup>25</sup> The pharmacological impacts of this herb called for its *in vitro* assessment.

*Warburgia salutaris* (Bertol.f) Chiov, belonging to *Canellaceae* family, known for their characteristic 'peppery-bark', found within Southern Region of African Continent.<sup>26-28</sup> Survey of literatures revealed that *W. salutaris* application in the treatment and management of some common ailments in communities with modern health care system with accessible and affordable medicine remained a common practice among the inhabitants, showing cultural and traditional acceptability as choiced herbs that can ease their physiological burden.<sup>26,27</sup> The stem bark in Zulu traditional health care is used to treat coughs, colds and other topical application such as sores and inflammatory disorders.<sup>29</sup> Documented pharmacological activities on *W. salutaris* revealed significant analgesic, decongestant, antiinflammatory, antiplasmodic and sedative properties<sup>30</sup>. Various drimane sesquiterpenes compounds and Iso-Mukaadial acetate have been derived *via* systematic isolation.<sup>29,31,32</sup> Nevertheless, a closer evaluation of these three South African herbs (*E. autumnalis*, *Z. mucronata* and *W. salutaris*) on *in vitro* free radical inhibitory activity is still not adequately documented in the scientific literatures, despite the feedback on their healing effects in Zulu traditional medication. To the best of our knowledge, evaluation regarding, cytotoxic and antioxidant activities of these South African medicinal plants (*E. autumnalis*, *Z. mucronata* and *W. salutaris*) are still left unvalidated. This study aimed to provide for the first time in comparison, the empirical proof for the traditional usage of the plants.

## MATERIALS AND METHODS

### Chemicals

Chemicals and other reagents used in the cause of the experiment were all of analytical grade procured from Sigma-Aldrich Company, Limited, South Africa, unless otherwise started.

### Collection of plant materials

*Warburgia salutaris* (stem bark), *Ziziphus mucronata* (stem bark) and *Eucomis autumnalis* (bulbs) are the investigative plants materials in the current research. Fresh part of interest of these herbs were collected in the Botanical garden under the supervision of University of KwaZulu-Natal, Pietermaritzburg Campus (29° 37' 30" S, 30° 24' 14" E), South Africa. Authentication was done by Mrs Alison Young with voucher specimen numbers; NU0043932, NU0049311, NU0048862. The plants were deposited at the Herbarium Unit, Botany Department of the same Institution.

### Preparation of the extracts

The dried plant parts (1kg) were sequentially extracted separately with methanol 72 h. Separation of the extracts mixture was done by the use of

Whatman No. 1 filter paper. Minimum volume of organic solvents were recovered at a decreased pressure of 40  $\pm$  2°C, with the aid of Heidolph rotary evaporatory machine. Concentrated extracts were dried under the fume hood overnight. The resultants yields were estimated.

## Antioxidant activity

### 2,2-diphenyl-1-picryl hydrazyl assay

Scavenging activity of the three plant extracts was evaluated using 2,2-diphenyl-1-picryl hydrazyl (DPPH) following a method previously described by Simelane *et al.*,<sup>13</sup> with minor modifications. Briefly, 2 mL of the methanolic extracts at concentrations range (20-100  $\mu$ g/mL) was added to equal volume of DPPH (2 mg) radical solution. The combined heterogenous solution was vigorously shaken and left upon standing for 30 to 60 min for proper colour visualization. Calibrations of absorbance values for the resultant solution was read at a wavelength of 517 nm.

### Superoxide anion(O<sub>2</sub><sup>-</sup>) assay

Reductive and inhibitory ability of *Z. mucronata*, *W. salutaris* and *E. autumnalis* on nitroblue tetrazolium (NBT) upon exposure to riboflavin-light generated NBT complex was investigated using the stepwise outlined procedure demonstrated by Nagai *et al.*,<sup>34</sup>. The reacting components including 0.02 mL of the methanolic extracts (20-100  $\mu$ g/L), 0.02 mL (50 mM, pH 10.5) of NaCO<sub>3</sub> buffer, 0.15% bovine serum albumin, 3 mM xanthine, 3Mm ethylenediaminetetraacetic acid (EDTA), 0.75 mM NBT were mixed. The combined solution was incubated at 25 °C for 20 min. The reaction was initiated and proceed faster on the addition of 0.02 mL of xanthine oxidase. Following the development of blue formazan observed for 20 min at 25 °C, is the stepwise introduction of 0.02 mL of 6 mM CuCl<sub>2</sub>. The absorbance values of the solvent system was calorimetrically determined at 560 nm.

### Nitric oxide (NO<sup>-</sup>) inhibitory assay

The production of nitric ion from sodium nitroprusside in water and oxygen at the physiological pH form the basis of this assay. Griess Illosvoy method of quantification was employed for the nitric oxide production.<sup>35</sup> Nitric oxide neutralizers competitively inhibit oxygen affinity and limit the concentration of nitric oxide production.<sup>36</sup> The reacting components include 3 mL out of the entire solution, comprising 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01M) with 0.5 mL of varying concentrations (20 - 100  $\mu$ g/L) of the methanolic extract of *Z. mucronata*, *W. salutaris* and *E. autumnalis*, mixed and incubated for 2h 30 min at room temperature. In addition to the solvent system above (0.5mL containing nitrite), 1 mL of sulphanic acid system (0.33% in 20% glacial acid) was pipetted forming a resultant solution. The solution was left to stand for 5 min at room temperature to give room for normal diazotization. The reaction was completely initiated at the instant of introducing 1mL of naphthylethylenediamine dihydrochloride (0.1%) in diffused light and was allowed to remain for 30 min. Absorbance at 540nm was calibrated.

### Hydroxyl radical (-OH) inhibitory assay

The hydroxylation of benzoic acid technique stated by Chung *et al.*,<sup>37</sup> was employed in this bio-assay experiment. 0.2 mL of solution containing 10 mM of Iron (II) sulphate heptahydrate, Ethylenediaminetetraacetic acid and DNA, phosphate buffer (pH 7.4; 0.1 M) and graded concentrations (20-100  $\mu$ g/L) of the methanolic extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis* were completely mixed. Addition of 200 $\mu$ L of hydrogen peroxide solution (10 Mm) to the reaction system was carefully done after which the solution was incubated for 2 h at 37 °C. 1mL of equal volume of TCA

(2.8% ) and TBA (1%) were incorporated into the mixture and heated to a boiling point in water bath for 10 min and were allowed to cool on ice. Calorimetric values at 520 nm of the solution system against the corresponding blanks were read.

### Reducing power assay

The outlined procedure for evaluating the reducing ability of sample reported by Oyaizu<sup>38</sup>, was adopted in this assay. Approximately, 2.5 mL of the varying concentrations (20-100 µg/ml) of the methanolic extract of *Z. mucronata*, *W. salutaris* and *E. autumnalis* were respectively added to equivalent volumes of 0.2M phosphate buffer at pH 6.6 and 1% potassium ferricyanide and incubated for 20 min at 50 °C. 2.5 mL of 10% Trichloroacetic was added to the solution and centrifuged at 1000 rpm for 10 min. Upon centrifugation, 2.5 mL of the upper layer of the reaction mixture was inoculated into an equal ratio of distilled water and FeCl<sub>3</sub> (0.5 mL, 0.1%). Measurement of absorbance was done at a wave length of 700 nm. The higher the absorbance value in this case the better the reductive capacity.

### Metal chelating activity

The method described by Decker and Welch<sup>39</sup> was used to evaluate the iron (II) chelating ability of the extracts. At graded concentrations (20-100 µg/L) of the methanolic extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis*, 0.5 mL of the organic solution was added to 1.6 mL of purified H<sub>2</sub>O and 0.05 mL of iron (II) chloride (2 mM) respectively. Initiation of reaction was done when 5 mM ferrozine (0.1 mL) was added at observation period of 30 secs. The entire reaction system was maintained at 25°C for 10 min, after evenly shaken on the orbital shaker. The absorbance values were determined at a corresponding wavelength of 562 nm.

### Lipid peroxidation inhibitory assay

Inhibitory activity of the extracts against induction of peroxidative reaction by Fe<sup>2+</sup>/Ascorbate complex was experimentally evaluated on liver obtained from Sprague-Dawley rats with average weight of 180 g. Perfusion and homogenization (1:10 w/v) employing 120 mM potassium chloride, 50 mM phosphate buffer (pH 7.4). Centrifugation (700 ×g, 10 min, at 4 °C) of the supernatant was carried out at a low temperature (-20 °C). Lowry *et al.*,<sup>40</sup> methodology of protein quantification was adopted, after which the protein contents have been carefully calibrated up to a concentration of 10 µg/mL. The composite mixture of the reactants comprising equal volume of (0.1 mL) of the diluted homogenate, Tris-hydrochloric acid buffer (20 mM pH 7), potassium chloride (30 mM), and including varying concentrations (20 – 100 µg/mL) of the methanolic extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis*, were incubated for 5 min which was maintained at 37°C. Addition AA/Fe<sup>2+</sup> (0.1 mL; 0.1 mM) to the reacting system initiated peroxidative reaction after incubating at 37°C for 30 min. Lipid peroxide values were determined by the thiobarbituric acid method at a wavelength of 532 nm.<sup>41</sup>

### MTT assay procedure

Cytotoxicity evaluation of methanolic extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis*, employing 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was conducted on HEK293 cells (a non-cancerous cell) in 96 well micro plates reader in accordance with the method clearly outlined by Mosmann<sup>42</sup> with some modifications. Incubation of the varying concentrations (20-100 µg/mL) of the methanolic extract, following the cell line, HEK293 in an ideal experimental condition composed of 5% CO<sub>2</sub> at 37°C for an observation period of 48h. The existing medium was carefully separated, while the addition of the new medium (100 µg/mL) and 5 mg/mL of MTT solution in PBS to the various wells on the plates prior to final incubation period. The solution containing MTT were

incubated alongside with cells at 37°C for 4h. 200 µL of DMSO was introduced into the well in order to solubilize formazan salt upon the removal of both the MTT solution and the medium. Absorbance at the wavelength of 570 nm was calibrated by 96A microplate reader. The percentage cell viability was calculated with the statistic shown below:

$$\% \text{ Cell viability} = \frac{\text{Asorbance of sample} \times 100}{\text{Asorbance of control}}$$

### Assay on DNA protection

Evaluation on the ability of *Z. mucronata*, *W. salutaris* and *E. autumnalis* extracts to protect a transformed variant of DNA (pET151/D-TOPO plasmid) extracted from *Escherichia coli* against free radicals damaging agent from Fenton's reagent composition as previously performed but with specific modifications.<sup>43</sup> The composite reaction mixture were made up of 10 µL of plasmid DNA, 2.5 µL of Fenton's reagent composition (H<sub>2</sub>O<sub>2</sub> (30%), Ascorbic acid (50 Mm) with 80 Mm of iron (III) chloride] and concentration of the methanolic extracts (in methanol and DMSO separately) at 20 µg/mL inclusively. The total volume of the reaction mixture was diluted to 20 µL with distilled water. The mixture were incubated for 30 min at 37°C. Addition of 5 µL of the binding dye (bromophenol blue; 0.25% composed of 50% glycerol) were followed immediately after the incubation period. 20 µL of each sample was loaded on agarose gel system (0.8%) in 10× TAE buffer solution, stained with 5 µL of ethidium bromide for brilliant visualization. Electrophoretic mobility of the agarose gel composition was carried out for 60 min at 90 volts. The nicked and native DNA were visualized using the G:BOX F<sub>3</sub> Gene System image visualizing technique for proper inference.

### Statistical analysis

The data were represented in mean ± standard error of the mean of three replicates (n=3). The inhibitory concentration with 50% values of inhibition (IC<sub>50</sub>) was calculated from the graph of percentage inhibition and later subjected to InStat Software, Bonferroni multiple comparison test, Graph Pad Prism 5, InStat Software (San Diego, USA%). Inhibition of each parameter was estimated using the following fomular:

$$\% \text{ inhibition} = [1 - (\text{Sample})/(\text{Control})] \times 100$$

## RESULTS

### Scavenging activity

*Ziziphus mucronata*, *Warburgia salutaris*, and *Eucomis. autumnalis* were assessed for *in vitro* free radical neutralizing power on DPPH, O<sub>2</sub><sup>-</sup>, ·OH, NO<sup>-</sup> as depicted in Table 1. Ascorbic acid (AA) and Butylated hydroxytoluene (BHT) were selected as model molecules in this *in vitro* evaluation. Comparatively with the standard synthetic antioxidant that were employed in the assessment, the plant extracts exhibited viable inhibitory ability on the radicals (Table 1).

## DISCUSSION

The scavenging influence of *Z. mucronata* on DPPH at varying concentrations range (20-100 µg/mL) were reasonably significant with the highest percentage level of inhibition (84.66%) at 100 µg/mL. The IC<sub>50</sub> value was 12.88 µg/mL which was relatively lower than the synthetic antioxidant (AA with IC<sub>50</sub> value of 3.16 µg/mL and BHT, IC<sub>50</sub> value, 0.15 µg/mL). *W. salutaris* also displayed reasonable level of inhibitory activity at these concentrations. The scavenging effects (93.62%) was on an increase at the peak concentration, with IC50 value, 20.32 µg/mL. Extract of *E. autumnalis* has IC<sub>50</sub> value of 57.02 µg/mL, with 60.12% inhibition at the highest concentration.

The inhibitory effects brought about by the scavenging activity of the crude extract could possibly due to their inherent chemical substances

**Table 1: IC<sub>50</sub> (µg/mL) and LC<sub>50</sub> (µg/mL) values of *Z. mucronata*, *W. salutaris*, *E. autumnalis* on different assay models [DPPH, Superoxide anion (O<sub>2</sub><sup>-</sup>), Hydroxyl (·OH), Nitric oxide (NO<sup>-</sup>), Lipid peroxidation (LP) and Cytotoxicity (CYT)].**

	IC <sub>50</sub> (µg/mL)					
	DPPH	(O <sub>2</sub> <sup>-</sup> )	(·OH)	(NO <sup>-</sup> )	LP	CYT. (LC <sub>50</sub> )
ZIZ	12.88 ± 0.46	43.95 ± 0.23	67.45 ± 0.28	55.46 ± 0.32	41.40 ± 1.53	5902.01
WB	20.32 ± 3.7	42.85 ± 3.23	81.28 ± 0.44	2.09 ± 0.15	21.78 ± 0.72	3118.89
EUM	57.02 ± 0.62	75.86 ± 0.5	>100	>100	>100	6011.74
A. Acid	3.16 ± 0.32	53.2 ± 0.9	>100	30.20 ± 0.34	7.26 ± 0.54	25234.81
BHT	0.15 ± 0.27	71.12 ± 0.24	65.92 ± 0.18	>100	13.18 ± 0.27	

Values are mean ± SEM (n=3) of IC<sub>50</sub> values of *Z. mucronata* (ZIZ), *W. salutaris* (WB), *E. autumnalis* (EUM), Ascorbic Acid (AA) and Butylated hydroxytoluene (BHT), respectively.

that act as radical quencher and can freely transfer electron to the counterpart molecules during the initiation and propagatory stage of these ROS.<sup>33,44-47</sup>

Inhibitory activity of the crude form (extracts) of the plants were evaluated on the superoxide and hydroxyl anion radicals. These radicals are thought to have beneficial and damaging impact on the important biological molecules.<sup>9</sup> The scavenging assay experiment on superoxide for the three extracts in the present study, (Table 1) revealed high level of inhibition on superoxide propagation corresponding with the IC<sub>50</sub> values to *Z. mucronata* (43.95 µg/mL), *W. salutaris*, (42.85 µg/mL) and *E. autumnalis* (75.86 µg/mL) and that of the standards agents (AA and BHT) used in the comparative measurement of the extent of the inhibition on the radical oxide. Hydroxyl radical has been reported as the most active interms of reactivity among the radical species and are known with the frequent attack on the vital macro-molecular blocks.<sup>15</sup> *Z. mucronata* and *W. salutaris* estimated IC<sub>50</sub> values were 67.45 µg/mL and 81.28 µg/mL. The IC<sub>50</sub> values of *E. autumnalis* was experimentally greater than the peak concentration selected in the course of the investigation a mark of weak inhibitory action on the hydroxide radical ion. The potency of *Z. mucronata* and *W. salutaris* in view of their hydroxide radical scavenging property were in close comparison with the standard synthetic molecule interms of their ability to prevent the progression of this hydroxyl anion. It is vivid that these herbs are inherent with natural molecules that are thought to aid in limiting the propagation of free radicals anions and increase the dietary antioxidants value *in vivo*.

The *de novo* synthesis and metabolism of nitric oxide is being used to monitor and evaluate its amount in the biological system.<sup>48</sup> The scavenging activity of the crude extracts form of *Z. mucronata*, *W. salutaris* and *E. autumnalis* with the standard synthetic antioxidants are presented in Table 1. *W. salutaris* exhibited potent inhibitory ability to the propagatory nature of the nitric oxide (IC<sub>50</sub> value of 2.09 µg/mL) better than the extracts of *Z. mucronata* and *E. autumnalis* and the standard antioxidant employed combined in this study. The scavenging power of *Z. mucronata* was moderately lower (IC<sub>50</sub> value of 55.46 µg/mL) relative to AA (30.20 µg/mL) and BHT with no inhibitory activity on the nitric oxide under the condition of this investigation, and as such similar in action to *E. autumnalis*. Evaluation at the level of both *in vivo* and *in vitro* nitric ion is vital to the cellular activities.<sup>49,50</sup> Thus, it is however worthy to postulate in clear terms that, *W. salutaris* is the strongest scavenger of free radical of all the extracts in this assessment including the synthetic antioxidant molecules. At any rate, the extracts significantly prevent the dominant and propagation of nitric ion at different capacity. In the overall, the inhibitory influence of *W. salutaris*, *Z. mucronata* and *E. autumnalis* might probably attributed to the presence of bioactive molecules reside in the plant extracts and are the main factors eliciting these pharmacological phenomenal.

### Lipid peroxidation assay

Plant extract have been reported to exert inhibitory action on Fe<sup>2+</sup>-induced lipid peroxidation in tissues homogenates *in vitro* and *in vivo* with a significant reduction in malondialdehyde (MDA) contents

owing to inbuilt antioxidant properties exerted by phenolic moiety, with the ability to scavenge DPPH radical and potent reductive ability on any radical assay experiment.<sup>47,51,52</sup> The plant's extract in their crude states have demonstrated variously, anti-lipid peroxidative ability by different modes (Table 1). *Z. mucronata* and *W. salutaris* exhibited 64.824%, 77.286% inhibition at the highest concentration (100 µg/mL). *E. autumnalis*, has no degree of antiperoxidative action in this assay at the vary concentration (20-100 µg/mL) under the condition of our investigation. The IC<sub>50</sub> values of *Z. mucronata* and *W. salutaris* were experimentally estimated at 41.40 µg/mL and 21.78 µg/mL. While the synthetic antioxidants; AA and BHT with IC<sub>50</sub> values of 7.261µg/mL and 13.183 µg/mL respectively, at same concentration. The anti-lipid peroxidative activity shown so far by *Z. mucronata* and *W. salutaris* in their crude states have placed these medicinal plants the more as important promising agents that could prevent cellular oxidation of the bio-membrane.

### Reducing power

Reducing capacity of any molecule be it natural or synthetic is no doubt depends on the nature of the molecule and the inherent property to prevent free radical propagation through the transferring of proton to the radical species<sup>53</sup> and inhibition of the occurrence of lipid peroxidative phenomenon via certain flavonoids or phenolics constituents.<sup>47</sup> Considering the results presented in Figure 1A, the percentages reductive capacity of *Z. mucronata*, *W. salutaris* and *E. autumnalis* were given as 71.76%, 76.69% and 61.11% respectively, at the maximum concentration (100 µg/mL) compared to the synthetic antioxidants molecules used for the assesment in this assay, BHT (65.61%) and AA (51.67%) at same concentration (20-100 µg/mL) Hence, reducing action exerted by crude extracts in this comparative study relative to the standard agents employed is a confirmation that natural products has inbuilt capacity to limit oxidation of biomolecule.<sup>53</sup>

### Metal chelating activity

It has been report in the literatures that most natural occurring antioxidant are good chelators of ferrous ions but mostly demonstrate weak hydroxyl radicals scavenging activity in most experimental studies.<sup>54</sup> The binding affinity of ferrous ion and free radicals neutralizing activity of a reduced molecules is a reliable marker of metal chelating property.<sup>55</sup> There was moderate to high chelating activity observed from the crude extracts investigated in this assay. *Z. mucronata* chelated upto 77.30%, compared with EDTA (82.07%) and CA (64.17%) at the concentration of 100 µg/mL (Figure 1B). There was minimum chelating effects observed in the manner in which both *W. salutaris* (68.867%) and *E. autumnalis* (64.836%) at same concentration compared to the investigative standard employed. The results is in complete agreement with the observation earlier cited in the literary study that antioxidant mechanisms are synergistic and complementary in action.<sup>55</sup>

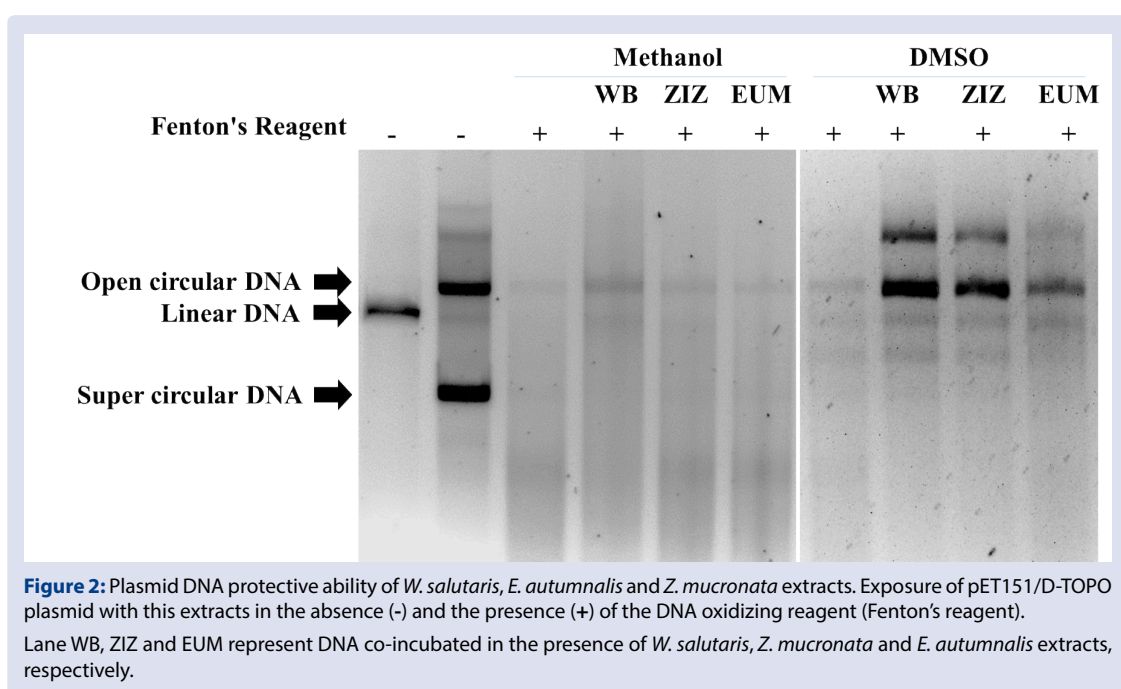
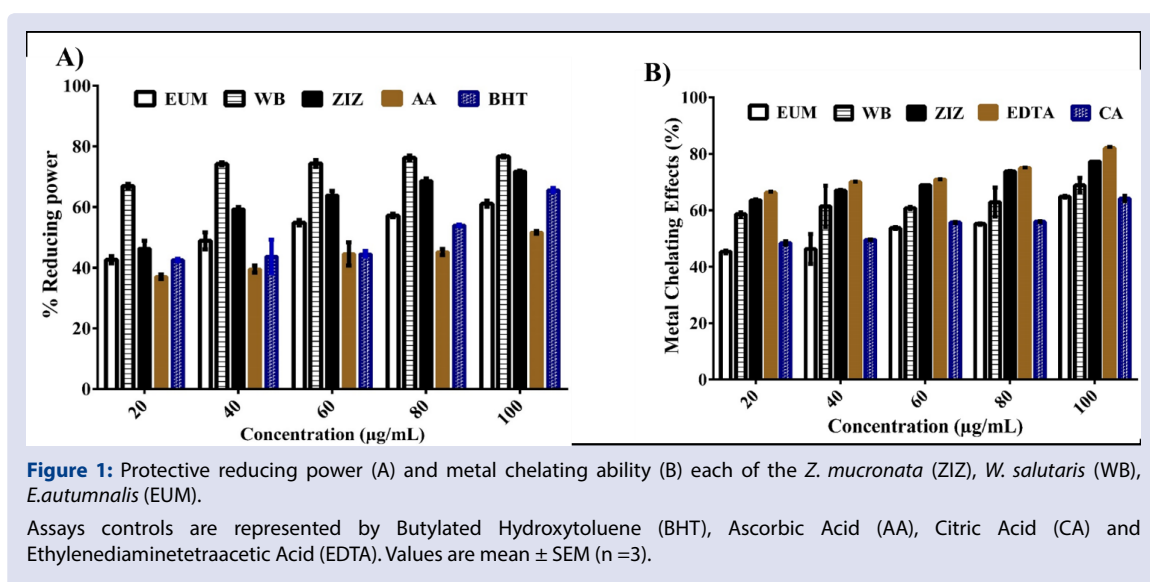
## Cytotoxicity

Sigidi *et al.*,<sup>56</sup> earlier postulated that in order for any natural product of herbal origin to be clinically relevance, there must be preferential lethality that is aiming at the host system in question, and hence interference with metabolic activities with bearable level of lethality. Cytotoxicity evaluation of methanolic extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis*, using MTT assay procedure on HEK293 cell line and the experimental control counterpart employed, ascorbic acid respectively are represented in Table 1. Evaluation of percentage lethality of these extracts on HEK293 cells resulted to low toxic concentration. Lethal concentration of these extracts at the peak varying concentration were estimated as 5902.01  $\mu\text{g/mL}$ , 3118.89  $\mu\text{g/mL}$  and 6011.74  $\mu\text{g/mL}$  for *Z. mucronata*, *W. salutaris* and *E. autumnalis* respectively. The low level of cytotoxicity could probably not associated with any deliterous effects on the cell survival. The degree of toxicity exhibited by these extracts are some way related to the level of preference of these extracts on the cell line employed.<sup>56</sup> It is therefore, imperative to note that the experimental herbs have wide range of safety marging of toxicity, with minimal

amount of lethal compounds, but when cautiously administered, they are therapeutically viable.

## DNA protection against oxidative stressing agent

In this study we have been able to establish for the first time on these plants, the significant correlation between protective ability of DNA and that of the free radical ( $\cdot\text{OH}$ ) scavenging strength of the methanolic extracts form of *Z. mucronata*, *W. salutaris* and *E. autumnalis* (Figures 2). The results of the *in vitro* assay under this assessment tally in many ways with the DNA protective ability against hydroxyl radical intoxication in an extract dependent phenomenon. At dose of 20  $\mu\text{g/mL}$ , *W. salutaris* exhibited better protective power slightly higher than *Z. mucronata* and farther much higher than *E. autumnalis* as against the digested DNA with restriction enzyme (BamH1) and normal-undigested DNA. The conferment of protection on DNA against any damaging agent is an indicator of reiparement and restoration from the nicked conformation (linear) of the DNA from pET151/D-TOPO variant to circular form (native form). This observation, has open up a new area of application in



biomedical science in the arena of using DNA protection as a yardstick in the evaluation of antioxidant status of medicinal plants and vice versa. In the past, Golla and Bhimathati reported that *Desmostachya bipinnata* L. Stapf extract at 50 µg/mL scavenged and blocked oxidative damaging effects of hydrogen peroxide from Fenton's reagent and reduced iron (III) component of the stressing agent imposed on DNA.<sup>57</sup>

The impact of the hydroxyl radical agent on the plasmid DNA is thought to be through induction of oxidative fracture on the Guanine-Cytosine base pair in the DNA strands to a different conformation with no biological significance (linear or inactive form). Interaction of linear form of DNA with hydroxyl radical in the reaction compartment progressively advances to fracture strands. The nitrogenous components of the DNA are oxidatively attacked by the hydroxyl anion with strong affinity and create in the nucleotide radical ions (base anion and sugar anion containing negatively charged phosphate bond). Thus, charged base radical anion combined with sugar counterpart resulting in an unstable complementary structure of the nucleotide molecule to separate.<sup>58</sup> Evidence abound from the data generated in the ongoing work that *Z. mucronata*, *W. salutaris* and *E. autumnalis* competently effective in protecting by limiting radical anion evolved from Fenton's reaction. Nevertheless, the intensity and appearance of the damaged DNA diminishes from *E. autumnalis*, *Z. mucronata* and *W. salutaris*. The protective nature of these extracts on DNA against free radical oxidative breakage is synonymous to the availability of primary metabolites of the family of polyphenolics and flavonoids known for neutralizing, stabilizing and limiting free radical molecules *in vitro* and *in vivo*.<sup>13,57,59</sup>

Interestingly, organic solvents exhibit various preferences in order to enhance protection to DNA against oxidizing agent and prevent DNA nicking effects. Organic liquid are known to avert direct interaction of the charged components of DNA with the iron (II) ion and hydroxyl anion from Fenton's reagents. DMSO has been described to have strong affinity in scavenging radical ion in the previous DNA nicked protection assay.<sup>60</sup> It is probable that the DMSO solubilized extracts completely chelate iron (II) and neutralize hydroxyl radical ion released by Fenton's reagent readily and better of than that methanol solvated extracts.

Thus, the demonstration by *Z. mucronata*, *W. salutaris* and *E. autumnalis* specifically, regarding the shielding capacity of DNA molecules from toxic chemicals, is a pointer to the fact that they are tested candidates in the treatment of oxidative associated diseases and promising agents in the treatment of molecular and genetic diseases.

## CONCLUSION

*Z. mucronata*, *W. salutaris* and *E. autumnalis* are indigenous medicinal plants domiciled in South Africa. They have demonstrated potent scavenging index *in vitro* and have even demonstrated competent shielding of DNA in the face of oxidative initiating and damaging molecule, DNA protection was concluded to be plant extract specific and potentially phyto-dependent. Nonetheless, reliably, these herbs showed potent activities against free radical propagation and as such could as well serve as alternative therapy in the biomedical world and promising agent in the therapeutic approach in the treatment of free radical associated diseases. To this end, the present study has justified the continuous participation of indigenous herbs as a complementary agent in treatment regimen. Hence, assessment of these plants and their derivatives in *in vivo* model experiment is worthy of investigation.

## COMPETING INTEREST

All Authors declare that no form of conflicting interest in this study.

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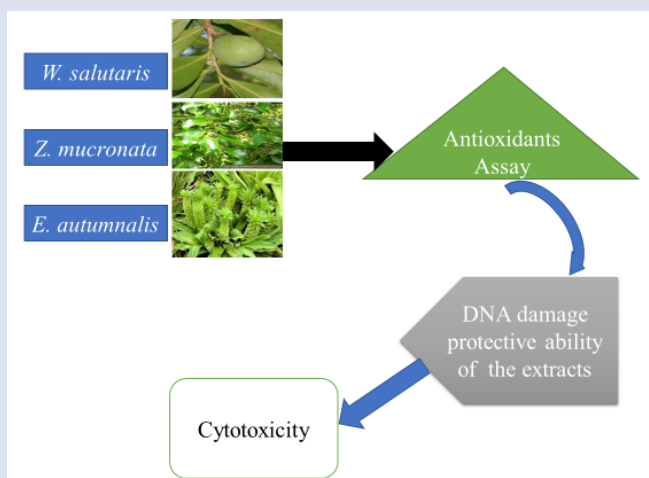
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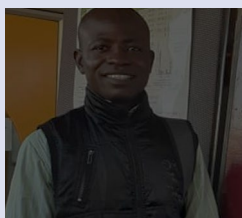
## GRAPHICAL ABSTRACT



## SUMMARY

The study elucidated that extracts of *Z. mucronata*, *W. salutaris* and *E. autumnalis* plants can potentially arrest free radical molecules through scavenging mechanism to prevent oxidative diseases.

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Mr. Ayeni research area is in the medicinal value of indigenous plants. He is currently interested in the isolation and characterization of active compounds that may be explore in the development of new drugs for liver diseases and other free radical related diseases. He is currently a staff member of Kogi State University, Anyigba, Nigeria.

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