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THE ANTICANCER, ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONCENTRATION OF ALOE FEROX MILL. LEAF EXTRACTS

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ABSTRACT

Aloe ferox is widely used in South Africa for its anti-inflammatory, laxative, antibacterial and antifungal activity. This study was done to screen A. ferox leaves for anticancer properties, antioxidant activity and to determine its phenolic content in gallic acid equivalents (GAE). The sulfurphodamine B (SRB) assay method was used to screen methanolic, and dichloromethane extracts against the MC7 (breast cancer), HCT116 (colon cancer) and PC3 (prostate cancer) cell lines for anticancer activity, using etoposide as the control. The stable radical 2, 2-diphenylpicrylhydrazyl (DPPH) was used to test the radical scavenging (antioxidant) activity of the plant extracts and compared to that of ascorbic acid. The Folin Ciocalteu method was used to determine the phenolic content of the methanol, dichloromethane and aqueous extracts against a gallic acid standard. The methanolic extracts showed antioxidant capacity with an EC50 of 0.865 mg/ml and thus is a potential source of natural antioxidants. The methanol extract (5 mg/ml) of Aloe ferox had the highest polyphenolic content with a GAE of 0.726.

KEYWORDS

Aloe ferox, anticancer activity, antioxidant, phenolic content.

INTRODUCTION

There is a growing interest across the world for the discovery of new drugs, especially from natural sources such as plants. They present a less expensive and non-toxic option for health care. The increased use of plants worldwide can be credited to high unemployment rates, expensive healthcare and life in general[1,2]. The development of resistance to synthetic drugs by different organisms as well as the toxicity of these drugs also contributes to the major interest in plants as potential sources of different therapeutic drugs. Due to the alarming high rates of uncontrolled harvesting, some of the South African plant species stay threatened and may become extinct. This can happen before their potential as useful sources of drugs can be discovered[2].

Chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases fuelled by the ways of life of the modern generation are affecting the entire world. Most of the common diseases except, bacterial and fungal diseases result from oxidative stress damage from reactive oxygen species (ROS) and reactive nitrogen species (RNS)[3,4]. These ROS and RNS entities can only be eradicated by antioxidant compounds found in high quantities in fruits, vegetables and other plants. The antioxidants present in plants can be used to replace the synthetic drugs by high unemployment rates, expensive healthcare and life in general[1,2]. The development of resistance to synthetic drugs by different organisms as well as the toxicity of these drugs also contributes to the major interest in plants as potential sources of different therapeutic drugs. Due to the alarming high rates of uncontrolled harvesting, some of the South African plant species stay threatened and may become extinct. This can happen before their potential as useful sources of drugs can be discovered[2].

The antioxidants present in plants can be used to replace the possibly carcinogenic and harmful synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA)[5,6]. Polyphenols which also have antimutagenic and anticancer activity are the most commonly studied antioxidants[6]. It is due to this activity and their abundance in medicinal plants that ethnobotany has become the centre of focus for most researchers.

The Asphodelaceae family has polyphenol-rich species. Only a few of these species have been investigated[7] and most of the scientific evaluation has only been done on Aloe vera which is highly used in cosmetics. Aloe vera has antisepctic, wound healing, antitumor and antidiabetic properties. Due to the diversity of the genus and their abundance in South Africa, the local communities have decided to use which ever species is available in their region[8].

Although plant species such as Aloe ferox Mill. are widely used and have many documented medicinal uses, their use, role as well as efficiency is still to be studied and validated. Aloe ferox is locally used for its antibacterial, antifungal as well as laxative properties[7]. The Aloe ferox leaf gel alone has been shown to contain polyphenols, indoles and alkaloids which all have antioxidant capacity[8]. These findings make Aloe ferox a possible source of antitumor agents. In this study different extracts of the Aloe ferox leaf were screened for anticancer and antioxidant activities. The phytochemical screening and total polyphenol determination were also carried out on the extracts.

MATERIALS AND METHODS

Sample preparation

Aloe ferox was purchased from a nursery in Pretoria, South Africa. A voucher specimen (RM001) was kept for future reference in the Biomedical Technology research laboratory at the Central University of Technology. The leaves were washed, oven dried at 40°C, pulverized. Ten grams of the powdered plant material was then soaked separately in 150 ml of different solvents; purified water, methanol, and dichloromethane and shaken for 72 hours, after which the extracts were filtered. The methanol and dichloromethane solvents were then removed through rotary evaporation at 35°C and the water extracts were lyophilized.

Before screening for different activities, the extracts were redissolved into the appropriate solvents in accordance to the required concentrations and volumes for each assay.
**Phytochemical screening**

Different qualitative methods from De et al.[9] were used to determine the phytochemical make up of Aloe ferox extract. The extracts were screened for carbohydrates, proteins, steroids, saponins, alkaloids and flavonoids.

**Test for carbohydrates: Fehling’s test**

To 2 ml of the sample, 2 ml of Fehling’s reagent A and B were added. The solution was then shaken and boiled. The formation of a brick-red precipitate (cuprous oxide) indicates the presence of reducing sugars.

**Test for steroids: Libermann- Buchard test**

A few drops of acetic anhydride were added to treat the extract, the mixture boiled and then cooled. Sulphuric acid was added to the mixture drop by drop to the sides of the test tube. The presence of steroids was indicated by formation of a brown ring at the junction of the two layers with the upper layer turning green. Formation of a deep red colour at the upper layer indicated the presence of triterpenoids.

**Test for saponins: The foam test**

Two millilitres of the extract were mixed with 2 ml of distilled water and the solution was shaken vigorously. The formation of persistent foam was an indication for the presence of saponins in the extract.

**Test for alkaloids: Dragendorff’s test**

The extract was mixed with a few ml of Dragendorff’s reagent. Alkaloids give a reddish brown precipitate.

**Test for flavonoids: Shinoda’s test**

Flavonoids give a pink scarlet, green or a green-blue colour after addition of a few fragments of magnesium ribbons and a drop-wise addition of concentrated hydrochloric acid.

**Anticancer activity screening**

The human cancer cell lines MCF7 (breast cancer) and PC3 (prostate cancer) were obtained from the National Cancer Institute (NCI) in Pretoria, South Africa. The colon cancer cell line (HCT116) was obtained from European Collection of Cell Cultures (ECACC). The cell lines were maintained in a monolayer cell culture at 37ºC, 5% CO2, 95% AIR and 100% relative humidity.

Cultures (ECACC) and NCI in Pretoria, South Africa. The colon cancer cell lines (HCT116) was obtained from European Collection of Cell Cultures (ECACC). The cell lines were maintained in a monolayer cell culture at 37ºC, 5% CO2, 95% AIR and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2mM L-glutamine and 5 µg/ml gentamicin.

The Sulforhodamine B (SRB) assay method by Monks and colleagues[8] was used to test the cancer growth inhibitory effects of the Aloe ferox extracts against cancer cell lines. The cells were inoculated on 96 well microtiter plates at plating densities of 3-10,000 cells per well. They were then incubated at 37°C for 24 hours after which one plate was fixed with TCA. This plate was labelled the (T0) and represented the measurement of cells at room temperature. The test extracts at different concentrations (6.25-100µg/ml) were added to the other plates (Ti). The plates were then incubated for 48 h after which cold 50% trichloroacetic acid was used to fix viable cells to the bottom of each well. The cells were then washed, dried and dyed with SRB. Optical density measurements on a multiwell spectrophotometer were used to calculate the net cell growth percentage at 540nm. The following equations were used to calculate the percentage cell growth:

\[
\frac{(Ti - T0)}{(C - T0)} \times 100 \text{ for concentrations at which Ti < T0.}
\]

The biological activities of the extracts were reported as Total Growth Inhibition (TGI) and divided into 4 categories as shown in Table 1. Cells without any drug/extract added were used as the control, the blank was complete medium without any cells and the Etoposide was used as the standard.

**Antioxidant activity screening**

The method described by Brand-Williams[10] with some modifications was used to screen the plant extracts for antioxidant activity. A stable radical 2, 2- dipheny- 1- picrylhydrazyl (DPPH) at 0.1mM was used to investigate the free radical scavenging activity of the extracts. Ascorbic acid, an antioxidant was used as a standard. One hundred millilitres of methanolic solutions of the extract at various concentrations (0.078-2.5 mg/ml) was added to 2 ml of the DPPH solution. The mixtures were shaken vigorously and then incubated in the dark at room temperature for 1 hour. The absorbance of the solution was then measured with a Helios Epsilon, CAT: 9423UVE1000E ThermoSpectronic spectrometer at 517nm, and the percentage of antioxidant activity (AA) calculated using the following formula:

\[
AA\% = \frac{100 - ((Abssample–Absblank) x 100)}{Abscontrol}
\]

A solution of methanol (0.1 ml) and DPPH (2 ml) was used as the control and for the blank, methanol (1ml) was used. The EC50 for the sample was determined from a plotted graph of DPPH disappearance as a function of sample concentration.

**Total phenolic content**

The total phenolic content of the plant extracts was determined using the Folin- Ciocalteu method according to Waterhouse[12] with a few modifications. Gallic acid which is a strong antioxidant was used as the standard from which the phenolic concentration of the sample was determined and expressed as mg gallic acid equivalent (GAE). The sample (0.1 ml) was mixed with 46ml of distilled water in 50 ml volumetric flask and 1ml of 2N Folin- Ciocalteu reagent added. The mixture was then thoroughly mixed and allowed to react for 3 minutes after which it was topped up to 50 ml with sodium carbonate solution (2%). Then the solutions were allowed to incubate for 2 hours at room temperature, after which the absorbance of the solutions was measured at 760nm with the Helios Epsilon, CAT: 9423UVE1000E ThermoSpectronic spectrometer. This procedure was also followed for gallic acid to obtain the standard curve.

**RESULTS AND DISCUSSION**

**Phytochemical screening**

The aqueous plant extract showed no presence of proteins but was positive for carbohydrates, saponins, steroids, alkaloids as well as flavonoids. The presence of steroids and alkaloids strongly suggest that these extracts may have a potential in the production of anti-inflammatory or even anticancer agents. Any agent that has steroids and alkaloids is a strong contender...
Table 2: The biological activities of the extracts against the MCF7, HCT116 and PC3 cancer cell lines.

<table>
<thead>
<tr>
<th>Activities</th>
<th>Etoposide</th>
<th>Methanol extract</th>
<th>Dichloromethane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF7</td>
<td>HCT116</td>
<td>PC3</td>
</tr>
<tr>
<td>GI50</td>
<td>&lt;6.25</td>
<td>19.96</td>
<td>34.64</td>
</tr>
<tr>
<td>TGI</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>LC50</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>LC100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Figure 1: Effect of Methanol and dichloromethane extracts of *Aloe ferox* against the breast cancer (MCF7), colon cancer (HCT116) and prostate cancer (PC3) cell lines.

Figure 2: Free radical scavenging activity of the methanolic *A. ferox* extract.
often present in the form of saponins[14]. The pharmacological studies indicate the presence of steroids and triterpenoids which the present study show compounds that may support the findings against other cancer cell lines needs to be done. The inactivity mean the plant has no anti-cancer activity as more evaluation inactivity of the extracts against these certain cell lines does not have GI50 against the prostate and colon cancer cell lines. The inactivity of the extracts is not discouraging as the prostate cancer cell line which is much lower compared to the 86.72 µg/ml, the EC50 of the extract was 0.865 mg/ml compared to that of ascorbic acid which was 0.042 mg/ml.

**Anticancer activity screening**

According to the CSIR criteria (Table 1), the tested extracts (methyl and dichloromethane) are inactive against all the cell lines as indicated in Figures 1. The methyl extract exhibited inactivity against the colon, breast and the prostate cancer cell lines with the TGI of more than 100 µg/ml for all of them. Table 2 shows the results of the parameters measured. As shown the extract did show a lower GI50 of 86.56 µg/ml against the breast cancer cell line. The dichloromethane extract also showed no activity against the three cell lines with a TGI of more than 100 µg/ml. However the extract showed a 50% inhibitory concentration of 46.09 µg/ml against the prostate cancer cell line which is much lower compared to the 86.72 µg/ml needed to exert the same activity against the breast cancer cell line. The inactivity of the extracts is not discouraging as the etoposide was also inactive against the cell lines as shown on Figure 1. It required a concentration of more than 6.25 µg/ml to have GI50 against the prostate and colon cancer cell lines. The inactivity of the extracts against these certain cell lines does not mean the plant has no anti-cancer activity as more evaluation against other cancer cell lines needs to be done. The inactivity may also mean the concentrations used were low for both the etoposide and the test extract.

**Antioxidant activity**

The DPPH radical scavenging activity of the methanolic extract is shown in Table 3. The methanolic extract was able to reduce the stable free radical DPPH to a yellow coloured 1,1-diphenyl-2-picrylhydrazyl and this activity was thought to be a result of the hydrogen-donating ability of the extract compounds. The extracts have potent antioxidant activity which was compared to that of the standard (ascorbic acid), shown in Figure 2. The highest activity (101 ± 0.042%) was achieved at extract concentration of 2.5 mg/ml. The EC50 of the extract was 0.865 mg/ml compared to that of ascorbic acid which was 0.042 mg/ml.

**Total phenolic content**

Table 4 reports the results of total phenolics of the different extracts. The extracts were analysed at 5mg/ml against gallic acid. The phenolic content varied between the extracts. Methanol extracted more polyphenols than water or dichloromethane. The gallic acid equivalents of the estimated phenolic concentrations are arranged in ascending order in Table 4 and range from 0.340 ± 0.057 to 0.726 ± 0.032 mg/ml GAE.

**CONCLUSION**

In conclusion, the results of this study show that A. ferox has antioxidant capabilities which may be due to its polyphenolic composition. Methanol extracts more polyphenols than water and dichloromethane. The presence of alkaloids, steroids and flavonoids as proved by the phytochemical screening show that this plant has the potential to prevent development of symptoms of cardiovascular diseases, diabetes, cancer and other inflammatory diseases. They are the main constituents of most of the drugs currently in use for these diseases. Further testing especially using different cell lines for anticancer screening should be done to validate the plant’s use for cancer treatment. The lack of activity against cancer cells could also mean that the plant functions against cancer through other mechanisms that do not affect the cancer cells directly. Some of these mechanisms could be by easing pain, inflammation and other symptoms.

**REFERENCES**


### Table 3: Antioxidant activity of Aloe ferox leaf methanol extracts.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>% DPPH scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>101.25 ± 0.042</td>
</tr>
<tr>
<td>1.25</td>
<td>75.87 ± 0.386</td>
</tr>
<tr>
<td>0.625</td>
<td>52.98 ± 0.416</td>
</tr>
<tr>
<td>0.3125</td>
<td>29.92 ± 0.699</td>
</tr>
<tr>
<td>0.156</td>
<td>21.38 ± 0.252</td>
</tr>
<tr>
<td>0.078</td>
<td>12.61 ± 0.682</td>
</tr>
<tr>
<td>EC50</td>
<td>0.865 ± 0.783</td>
</tr>
<tr>
<td>Ascorbic acid EC50</td>
<td>0.042 ± 0.084</td>
</tr>
</tbody>
</table>

### Table 4: Total phenolic content of A. ferox extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance Mean</th>
<th>Polyphenol concentration (mg/ml GAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>0.069</td>
<td>0.340 ± 0.057</td>
</tr>
<tr>
<td>5mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichloromethane extract</td>
<td>0.109</td>
<td>0.550 ± 0.042</td>
</tr>
<tr>
<td>5mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>0.144</td>
<td>0.726 ± 0.032</td>
</tr>
<tr>
<td>5mg/ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


