IN VITRO ANTIOXIDANT ACTIVITY OF DICHLOROMETHANE: METHANOLIC LEAF AND STEM EXTRACTS OF PAPPEA CAPENSIS

David N. Ngai¹*, Michael N. Musila¹, Gervason A. Moriasi¹, Shadrack M. Njagi¹, James K. Kamau¹, Mathew Piero Ngugi¹ and Joan M. Njagi²

¹Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.
²Department of Environmental and Population Health, School of Public Health, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.

ABSTRACT
Oxidative stress is a metabolic disorder characterised by overproduction of the highly reactive free radicals that damage body tissues. Such radicals include the unstable and destructive reactive oxygen and nitrogen species among other free radicals. A reduction in the immunological antioxidant defences of the body coupled to a declining tissue antioxidant status is the main causes of oxidative stress. Too much free radical in the tissues ultimately triggers the onset of lifestyle diseases such as diabetes. The current tremendous rise in cases of diabetes mellitus and lack of its effective management strategies has made it necessary to search for antioxidant plant products that are more effective and have fewer side effects. Plant extracts are also known to be more affordable especially in the regions harbouring high populations of the plant in question and because of the fact that they are mostly used in their crude aqueous form. Many studies have been carried out on plants said to possess bioactive components and a good number of herbal products have been reported as possessing antioxidant properties. The current project was designed to evaluate the reducing potential of DCM: Methanolic stem bark and leaf extracts of P. capensis. The parameters used to assess the reducing potential of the two extracts are; 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, hydrogen peroxide scavenging activity, the total reducing power and phenolics and flavonoids content. The reducing power tests carried out showed that the stem bark extract of P. capensis had statistically significant
more reducing power activity compared to the leaf extract. The DPPH and H$_2$O$_2$ radical neutralising properties exhibited by the stem bark extract of *P. capensis* was significantly more in comparison to the leaf extract. The study established that the stem bark and leaf extracts of *P. capensis* had similar contents of phenolics, but the leaf extract proved to contain a higher level of flavonoids. This study demonstrated *in vitro* reducing potential of DCM: Methanolic leaf and stem bark extracts of *P. capensis*. The qualitative phytochemicals screening demonstrated the fact that terpenoids, phenolics, glycosides, alkaloids and flavonoids which are associated with antioxidant activity were present in the extracts.

**KEYWORDS:** Antioxidant, oxidative stress, phytochemicals.

**INTRODUCTION**

Elevated levels of oxidants are a significant contributing factor for the development and deterioration of oxidative stress and the associated diseases including diabetes mellitus. Following its onset, oxidative stress and the intermediate metabolites related to it are involved in up-regulation of many stress-sensitive signalling metabolic systems that cause inflammation, cytokine release and may lead to apoptosis. The transcriptional activators involved in these pathways include Poly ADP-Ribose Polymerase (PARP) together with transcription factor NF-κB. These highly potent molecules act as coactivators for proinflammation molecules such as Intracellular Adhesion Molecule-1 (ICAM-1), inducible Nitric Oxide Synthase (iNOS) and Major Histocompatibility Complex class II (MHC-II).[1]

Oxidative stress has been reported as one of the factors contributing to the increasing cases of insulin insensitivity, β-cell function suppression, disturbed glucose tolerance and increased risk for type 2 diabetes mellitus.[2] Oxidative stress is characterized by the persistent increase in oxidative reactions and elevated release of free radicals and reactive oxygen species leading to a drastic decrease in antioxidant defences and tissue antioxidant levels. Malondialdehyde (MDA) is a biomarker for oxidative stress that exists naturally in body tissues. Malondialdehyde can be used to estimate the degree of oxidative stress in tissues.[3] Other oxidative stress markers include the Advanced Glycation End products (AGEs), increased MDA levels, AGE Receptors (RAGEs), Advanced Oxidation Protein products (AOPs) and Advanced Lipidoxidation End products (ALEs) among others.[3]

Biochemicals that alleviate the oxidation of other molecules thus terminating the free radical series of reactions are referred to as antioxidants. Therefore, antioxidants are reducing agents
such as phenolics, minerals, thiols and vitamins A, C and E.\textsuperscript{[4]} The structure of phenolic compounds consists of at least one benzene ring with at least one hydroxyl group bound. For convenience, they are grouped into two groups, namely, non-flavonoids and flavonoids. Derived polyphenols which are derivatives of phenolic intermediate products are constituents of refined edibles such as black tea, naturally brewed red wine, cocoa and coffee.\textsuperscript{[5]}

Herbal preparations are believed to be safer alternatives and more effective compared to the conventional medicines in the management of ailments related to oxidative stress.\textsuperscript{[6]} There is a broad variety of antioxidant compounds synthesized by plants including carotenoids, lignans, flavonoids, isoflavones, isocatechins, flavones, ascorbic acid, anthocyanins, coumarins, catechins, polyphenols and \(\alpha\)-tocopherol to discourage the oxidative damage to their susceptible biomolecules. Basically, this is why some reducing agents are used in the formulation of some food products as additives to discourage oxidation of fats in the products.\textsuperscript{[7]}

The plants and spices that exhibit antioxidant potential include ginger, turmeric, sage, thyme, white pepper, nutmeg, chilli pepper and rosemary among others.\textsuperscript{[8]} A relationship has been reported between the phenolic content of herbal extracts and their antioxidant potential.\textsuperscript{[9]} Use of herbal teas and beverages with high phenolic levels has been shown to alleviate the possibility of heart diseases.\textsuperscript{[10]}

Herbal products have been used for dietary and medical purposes since immemorial times. For instance, acetylsalicylic acid was synthesized from salicin obtained from willow bark in 1897.\textsuperscript{[11]} Since then, herbal products have become noble sources of compounds used as important raw materials in medical research.\textsuperscript{[12]}

In Kenya, \textit{Pappea capensis} stem bark, leaf and seed oil are used traditionally to manage ringworms, baldness, eye infections and hyperglycaemia.\textsuperscript{[13]} The root of the plant is used as an enema and a purgative for cattle.\textsuperscript{[14]} This study was designed to establish the \textit{in vitro} reducing potential of DCM: Methanolic leaf and stem bark extracts of \textit{P. capensis}.

**METHODOLOGY AND MATERIALS**

**Collection and preparation of plant materials**

Fresh leaves and stem barks of \textit{P. capensis} were sourced from Mumini ward, Embu County, Kenya with the guidance of an experienced herbalist. The identification and authentication of
the plant was done and a sample specimen preserved at National Museums of Kenya herbarium in Nairobi, Kenya. The department of Biochemistry, Microbiology and Biotechnology, Kenyatta University provided the facilities for the study. After the materials were shade dried, they were ground into powder by use of a posho mill. The resulting powder was secured in sealed, dry paper bags.

**Extraction**

Half kg of *P. capensis* leaf or stem bark powder was stirred in one litre of a 1:1 mixture of MeOH and DCM and left to stand for two days. The extract was then decanted into a glass beaker before being sieved through cotton wool into another dry glass container. A rotary evaporator at 40°C was used to concentrate the filtrate and the extract stored in a fridge at 4°C ready for use.

**In vitro antioxidant assays**

**Total reducing power**

The ability of the extracts to reduce ferric ions to their ferrous form was determined using analytical grade vitamin C as a positive control. The protocol described by Oyaizu\(^1\) was followed. Five various concentrations of the DCM: Methanolic extract (10–50μg/ml) in 1.0 ml of distilled water (3 replicates), were put into phosphate buffer (2.5ml, 0.2M, pH 6.6).

A 2.5 ml aliquot of 1% potassium ferrocyanide was added and the mixture maintained at 50°C for 20 minutes. An aliquot of 2.5ml trichloroacetic acid (10%) was incorporated and the resulting solution centrifuged at 3,000 rpm for 10 minutes. A volume of 0.5ml of 0.1% FeCl\(_3\) was incorporated into the upper layer of the supernatant (2.5 ml) and OD taken at 700nm.

**DPPH radical reducing activity**

Plant extracts and ascorbic acid’s reducing properties of 1, 1-diphenyl-2-picrylhydrazyl were performed by methods proposed by Kuo.\(^2\) The test was carried out in a volume of 3 ml reaction mixture (3 replicates) comprising 0.9 ml of 50 mM Tris-HCl buffer (pH7.4), 2.0 ml of 0.1 mM DPPH-alcoholic solution and 0.1 ml of distilled H\(_2\)O or *P. capensis* extracts. The optical density of the solutions was taken at 517nm after twenty five minutes incubation in darkness at 25 degrees centigrade. The DPPH reducing activity was determined by the following equation:

\[
\text{DPPH Scavenging Activity} = \frac{Ac - As}{Ac} \times 100
\]
Where,

\[ Ac = \text{Absorbance of the control} \]
\[ As = \text{Optical density of the extracts and standard} \]

IC\(_{50}\) was determined by plotting percentage scavenging activity against concentration. It represents the concentration at which the test sample reduces 50% of the radicals.

**Hydrogen peroxide radical reducing activity**

A standard protocol described by Ruch was carried out to establish the potential of the plant extracts to reduce hydrogen peroxide radicals. Hydrogen peroxide (40mMol/L) solution was made in phosphate buffer (pH 7.4).\(^{[17]}\) 2.5ml of the extracts (3 replicates) at various strengths (0.1– 0.5 mg/ml) and hydrogen peroxide solution (0.6 ml) were mixed. Optical density was taken at 560nm after standing for ten minutes. The reducing potential of the samples was calculated by the formula of Ruch.\(^{[17]}\)

\[
\text{H2O2 Scavenging Activity} = \frac{Ac - As}{Ac} \times 100
\]

Where,

\[ Ac = \text{Optical density of the control} \]
\[ As = \text{Optical density of the extracts and standard} \]

A plot of percentage reducing activity against concentration was employed to evaluate IC\(_{50}\).

**Total phenolic concentration**

To quantify phenolics in the plant extracts and the standard, Folin-Ciocalteu reagent was used as per a protocol described by Lister and Wilson.\(^{[18]}\) A mixture of 2 ml of Na\(_2\)CO\(_3\) (7.5%, w/v), 5.0 ml of each sample (3 replicates) and 2.5 ml of 10% solution of Folin-Ciocalteu reagent was prepared. The mixture was maintained at 45°C for 15 min. The optical density of the samples was measured at 765nm against gallic acid as the standard using Spectronic-20 (Shimadzu). A standard curve was plotted and used to calculate the phenolics content of the extracts.

**Total flavonoid content**

To determine the concentration of the flavonoids in the extracts, a protocol described by Lamaison and Carnet was followed.\(^{[19]}\) A volume of 1.5 ml of plants extract and 2% AlCl\(_3\),6H\(_2\)O in methanol were mixed then shaken for five minutes. After 10 minutes at 25
degrees centigrade, optical density was taken at 430 nm. The standard rutin calibration curve was used. The flavonoids content of the extracts was assessed using an equation based on the calibration curve and stated in terms of milligrams of rutin equivalence per unit dry weight.

**Qualitative phytochemical screening**

*P. capensis* leaf and stem bark extracts were taken through a qualitative phytochemical composition evaluation to detect the presence or absence of some important phytochemicals. A protocol proposed by Harbone and Kotake[20,21] were used. The phytochemicals tested were cardiac glycosides, steroids, flavonoids, phenols, saponins, alkaloids and terpenoids. The above phytochemicals were specifically selected because they are known to possess antioxidant activity.

**Data management**

The data collected was analysed using Minitab statistical software and the results expressed as mean ± standard error of the mean. One-way analysis of variance (ANOVA) was applied to detect any statistical significance of difference among treatments followed by Tukey’s test for pairwise comparison of means of the various groups. The values of P ≤ 0.05 were considered significant.

**RESULTS**

**In vitro total reducing power**

The DCM: MeOH leaf and stem bark extracts of *P. capensis* demonstrated a dose-dependent reducing effect, which was demonstrated by the increase in absorbance with concentration (Table 1; Figure 1). The stem bark extract showed a significantly higher reducing power at concentrations of 20, 30 40 and 50µg/ml (p<0.05; Table 1). However, both extracts had similar reducing power activity at the lowest concentration of 10µg/ml (p>0.05; Table 1). On the other hand, the reducing power of ascorbic acid (the standard) was significantly more compared to the leaf and stem bark extracts of *P. capensis* (p<0.05; (Table 1).

**In vitro DPPH scavenging activity**

The DCM: MeOH leaf and stem bark extracts of *P. capensis* demonstrated dose-dependent DPPH scavenging activities, as demonstrated by the rise in proportionate scavenging activities with rising concentration of the extract (Table 2; Figure 2). The DPPH scavenging activity of the stem bark extract of *P. capensis* was significantly higher than that of the leaf extract at all the tested concentrations (p<0.05; Table 2). The standard (ascorbic acid)
displayed the highest DPPH scavenging activity, followed by the stem bark and the leaf extracts of *P. capensis* respectively (Table 2; Figure 2). The IC$_{50}$ for ascorbic acid, stem bark and leaf extracts were 2.94, 5.77 and 9.14µg/ml respectively (Table 2).

**In vitro hydrogen peroxide scavenging activity**
The DCM: MeOH leaf and stem bark extracts of *P. capensis* showed increasing scavenging activity of H$_2$O$_2$ as concentration was increased (Table 3; Figure 3). The stem bark extract showed an apparently greater hydrogen peroxide reducing activity compared to the leaf extract at all the strengths evaluated (p<0.05; Table 3). The standard (ascorbic acid) was the most potent hydrogen peroxide scavenger throughout. The IC$_{50}$ for ascorbic acid, stem bark extract and leaf extract were 0.03, 0.18 and 0.2 mg/ml respectively (Table 3).

**Total Phenolic content**
The total phenolics composition of the DCM: methanolic leaf and stem bark extracts of *P. capensis* were evaluated from the standard calibration curve. The stem bark extracts of *P. capensis* showed a significantly higher phenolics content at the levels of 0.1, 0.2, 0.3 and 0.4mg/ml (P<0.05; Table 4; Figure 4). However, the gallic acid equivalence of stem bark and leaf extracts of *P. capensis* at the concentration of 0.5mg/ml showed insignificant variability (P>0.05; Table 4).

**Total flavonoids content**
The total flavonoids content of the DCM: MeOH stem bark and leaf extracts of *P. capensis* were calculated from the standard rutin calibration curve. The flavonoids composition of the leaf extract was significantly greater than that of the stem bark extract at concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml. (P<0.05; Table 5; Figure 5).

**Phytochemical screening (qualitative)**
The results of qualitative phytochemical screening of the DCM: MeOH leaf extract of *P. capensis* revealed the presence of cardiac glycosides, flavonoids, saponins and phenolics. However, terpenoids, steroids and alkaloids were absent (Table 6). The phytochemicals found in the stem bark extract of *P. capensis* were terpenoids, flavonoids, alkaloids, steroids, cardiac glycosides, saponins and phenolics (Table 6).
### Tables and Figures

**Table 1: Total reducing power of DCM: MeOH extract of *P. capensis***

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ascorbic acid</th>
<th>Stem bark extract</th>
<th>Leaf extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μg/ml</td>
<td>0.28±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 μg/ml</td>
<td>0.43±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 μg/ml</td>
<td>0.52±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>40 μg/ml</td>
<td>0.63±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 μg/ml</td>
<td>0.62±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42±0.005&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM of triplicate readings. Values with the same letter are not significantly different by one-way ANOVA along the rows followed by Tukey’s test (P<0.05)

**Table 2: DPPH scavenging activity of DCM: MeOH extracts of *P. capensis***

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ascorbic acid</th>
<th>Stem bark extract</th>
<th>Leaf extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μg/ml</td>
<td>46.40±0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.82±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.06±0.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 μg/ml</td>
<td>66.62±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.91±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.72±0.68&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 μg/ml</td>
<td>82.44±0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.96±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.97±0.64&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>40 μg/ml</td>
<td>86.31±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.55±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.15±0.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 μg/ml</td>
<td>90.71±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.55±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.18±0.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>2.94</td>
<td>5.77</td>
<td>9.14</td>
</tr>
</tbody>
</table>

Values expressed mean ± SEM of triplicate readings. Values with the same letter are not significantly different by one-way ANOVA along the rows followed by Tukey’s test (P<0.05)
Table 3: Hydrogen peroxide scavenging activities of DCM: MeOH extracts of *P. capensis*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ascorbic acid</th>
<th>Stem bark extract</th>
<th>Leaf extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1µg/ml</td>
<td>44.42±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.08±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.89±0.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2µg/ml</td>
<td>60.61±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.23±0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.84±1.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3µg/ml</td>
<td>76.96±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.82±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.49±0.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3µg/ml</td>
<td>83.14±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.68±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.07±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5µg/ml</td>
<td>86.93±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.04±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.88±0.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0.03</td>
<td>0.18</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation for three replicate readings. Values with the same superscript letter are not significantly different by one-way ANOVA along the rows followed by Tukey’s test (p>0.05).

Table 4 Total Phenolic contents of DCM: MeOH extracts of *Pappea capensis*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Gallic acid equivalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf extract</td>
</tr>
<tr>
<td>0.1mg/ml</td>
<td>0.037±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2mg/ml</td>
<td>0.150±0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3mg/ml</td>
<td>0.217±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4mg/ml</td>
<td>0.257±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5mg/ml</td>
<td>0.300±0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values expressed as mean± SEM of three replicates. Values with the same superscript letter are not significantly different using unpaired student t test along the rows.
Table 5 Total flavonoids content of DCM: MeOH extracts of *P. capensis*

<table>
<thead>
<tr>
<th>concentration</th>
<th>Leaf extract</th>
<th>Stem bark extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2mg/ml</td>
<td>0.117±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.043±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4mg/ml</td>
<td>0.247±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.080±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.6mg/ml</td>
<td>0.320±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.120±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.8mg/ml</td>
<td>0.410±0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.153±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0mg/ml</td>
<td>0.570±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.173±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM of three replicates. Values with the same superscript letter are not significantly different using unpaired student t test along the rows.

Table 6 Qualitative Phytochemical composition of DCM: MeOH leaf and stem bark extracts of *P. capensis*

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Leaf extracts</th>
<th>Stem bark extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Present phytochemicals are denoted by (+) sign, while absent phytochemicals were denoted by (-) sign.

Figure 1 Total reducing power of DCM: MeOH extracts of *P. capensis*
Figure 2 DPPH scavenging activity of DCM: MeOH leaves and stem bark extracts of *P. capensis*

Figure 3 Hydrogen peroxide scavenging activity of DCM: MeOH leaves and stem bark extracts of *P. capensis*
DISCUSSION

The in vitro antioxidant assays portrayed *P. capensis* as a good source of potent antioxidant molecules. There are several mechanisms through which plant extracts exhibit their antioxidant activities. These include the ability to reduce metallic ions, decomposition of hydrogen peroxide, free radicals scavenging and prevention of chain reaction initiation among others.\[(22]\]

From the results of the in vitro reducing power, the stem bark extract had significantly higher reducing power in comparison to the leaf extract of *P. capensis*. The
increase in extract concentration (10-50μg/ml) of *P. capensis* resulted to increased reducing activity.

The antioxidants present in the leaf and stem bark extracts of *P. capensis* resulted in the lowering of the oxidation state of Fe$^{3+}$ to Fe$^{2+}$ by transfer of electrons from the extracts to the ferric ions and the reaction rate is directly proportional to the antioxidant capacity of the extract.\[^{[23]}\] The qualitative phytochemical screening tests indicated the presence of phenolics and flavonoids. According to Bravo, plants rich in these phytochemicals have antioxidant activities due to the nature of their chemical structures and their reduction-oxidation properties.\[^{[24]}\]

The DCM: MeOH extracts of *P. capensis* showed strong antioxidant activities against DPPH and hydrogen peroxide. The DPPH radical is widely used in measuring of antioxidant properties because the reaction takes place quite readily.\[^{[25]}\] The DPPH and hydrogen peroxide reducing activity exhibited by the stem bark extract of *P. capensis* was statistically significantly higher compared to the leaf extract. This difference can be discussed on the basis of the various phytochemicals found in the two extracts. The qualitative phytochemical screening of DCM: Methanolic stem bark and leaf extracts of *P. capensis* demonstrated that phenolics, flavonoids, saponins and cardiac glycosides were present. However, the stem bark extract had steroids, terpenoids and alkaloids, in addition. Furthermore, both the stem bark and the leaf had high phenolics content, but the leaf extract proved to be richer in flavonoids.

The reducing properties of the DCM: MeOH extracts of *P. capensis* could be as a result of bioactive molecular component(s) observed in the extracts. The fact that the results of DPPH and hydrogen peroxide scavenging activities exhibited a similar pattern dispels any doubts about the candidature of *P. capensis* leaf and stem bark extracts as antioxidants and potential remedies for oxidative stress.

Apparently, the high phytochemical content of *P. capensis* crude extracts is responsible for its antioxidant activity. For instance, most prooxidant species, such as singlet oxygen, and many reactive nitrogen species are neutralized by flavonoids present in the extracts.\[^{[24]}\] There is strong evidence to the fact that flavonoids neutralise ROS formation, potentiate and enhance antioxidant defences and chelate metallic elements involved in the free-radical generation.\[^{[26]}\]
The reducing capacity of the studied extracts is not associated with their phenolic and flavonoid contents only. They contain an assortment of different compounds with unique pharmacological activities and synergism. The presence of some other phytochemicals such as saponins and steroids, or even ascorbic acid, α-tocopherol and pigments could have had an impact on the observed reducing properties of the extracts too.\[27\]

The steroids act in a similar manner to hormones produced by the body’s adrenal glands to reduce stress caused by illnesses and injuries. Saponins have a direct antioxidant potential that decreases the risk of developing malignancy, diabetes and cardiac diseases. In modern times, some heart problems have been treated with purified extracts or synthetic derivatives of cardiac glycosides from plants leading to increased heart contractional forces which in turn increases cardiac output.\[28\]

The use of plant materials and dietary supplements with high antioxidant activity is increasing. Such substances include herbs, vegetables and cereals which are rich in phenolics. Phenolics are known to have high antioxidant potential. Antioxidants are essential for good health. They are, in recent times, being recognized by conventional modern physicians as a significant part of the daily diet. The importance of antioxidants intake has been demonstrated in antiglycative experimental models. In this model, phenolic compounds namely urolithins A and B, combined with pyrogallol, prohibited glycosylation of polypeptides with 37% and 44% efficiency at two different concentrations.\[29\]

**CONCLUSION**

The current study findings can be concluded by stating that the DCM: Methanolic leaf and stem extracts of *P. capensis* has antioxidant activity. Methanolic leaf and stem bark extracts of *P. capensis* may therefore be used in development herbal remedies for oxidative stress complications.

**Conflict of Interests**

The authors declare that there is no conflict of interests.

**ACKNOWLEDGEMENT**

We appreciate the Department of biochemistry, microbiology and biotechnology, Kenyatta University, for allowing us access to their facilities during the study.
REFERENCES


