Research Paper

In vitro anti-oxidative and anti-inflammatory properties of Viscum album.

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ABSTRACT

Reactive oxygen species play a large role in the pathogenesis of oxidative stress and inflammation leading to diseases such as metabolic and neurodegenerative disorders. Considering the importance of these two physio-pathological mechanisms mutually implicated in these diseases, the aim of this study evaluated the in vitro anti-oxidative and anti-inflammatory properties of Viscum album extracts (methanolic, hydroethanolic, aqueous and decoction). Folin-Ciocalteu, Aluminium chloride and acidified vanillin methods were used to quantify total phenols, flavonoids and tannins respectively. Antioxidant capacity of the extracts was investigated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2’-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Ferric-Reducing Antioxidant Power (FRAP) methods. The anti-inflammatory activity was performed using protein denaturation and proteinase inhibition assays. The results showed, high content of total phenolic, flavonoids and tannins in methanolic and hydroethanolic extracts. Similarly, these two extracts showed high antioxidant activity in DPPH, ABTS and FRAP. The results of the scavenging concentration 50 showed that, the higher antioxidant activity was found in methanolic extract with 0.23 mg/ml in DPPH and 0.159 mg/ml in ABTS. The strongest ferric reducing ability was presented by hydroethanolic extract (785.32 ± 0.03 mg AAE/g) followed by methanolic extract (766.52 ± 0.02 mg AAE/g). The results of this study also shown that, the methanolic and hydroethanolic extracts were effective in inhibiting proteinase and heat-induced albumin denaturation. The inhibitory concentration 50 of methanolic and hydroethanolic were found to be lower than aqueous extract and decoction with 40.70 and 43.89 µg/ml for protein denaturation and 54.87 and 80.89 µg/ml for proteinase inhibition respectively. From all the results above, Viscum album could be a promising source of antioxidant with a good anti-inflammatory activity which can be used to treat oxidative stress and inflammation related diseases.

Key words: Viscum album, antioxidant, anti-inflammatory, protein denaturation, proteinase, oxidative stress, inflammation.

INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radical \( \text{O}_2^- \), hydroxyl radicals (OH), and hydrogen peroxide (H\(_2\)O\(_2\)), play an important role in the pathogenesis of oxidative stress which can lead to several diseases such as inflammation, cardiovascular diseases, diabetes, neurodegenerative diseases, cancers, anaemia and ischemia (Bridge et al., 2014; Singh and Singh, 2008). Basically, the level of free radicals in a living system is controlled by a complex of antioxidant defence, minimizing oxidative damage of important biomolecules. In oxidative stress circumstances, the endogenous antioxidants are not capable to clear the increased levels of ROS, thus, leading to the damage of cellular macromolecules such as DNA, proteins and lipids (Amoussa et al., 2015; Hussain et al., 2016). Damage caused by ROS may be due to their attack on membrane lipids, intracellular proteins/enzymes, carbohydrates, and nuclear DNA in cells and tissues. These include undesirable oxidation causing membrane dysfunction, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation (Singh and Singh, 2008). Furthermore, the oxidized proteins, glycated products, and lipid peroxidation result to
inflammatory response. Inflammation is a primary physiological defence mechanism that helps to protect the body against infections, toxins, allergens, or other harmful stimuli (Hossain et al., 2012). It is a complex process, often associated with pain and involved events such as: increased vascular permeability, increased protein denaturation and membrane damage (Umapathy et al., 2010).

Most biological proteins lose their biological function when denatured. Denaturation of protein is a well-documented cause of inflammation (Anoop et al., 2015). Evidences indicated that oxidative stress plays a pathogenic role in chronic inflammatory diseases (Hussain et al., 2016). As inflammation and oxidative stress are mutually implicated in several diseases, the search for compounds that can remedy these two problems are of importance. Several synthetic antioxidants and anti-inflammatory drugs have been developed to alleviate oxidative stress and inflammation, respectively (Ravipati et al., 2012). However, factors such as the high cost, unavailability and side effects of these compounds remain a limitation in the fight for the decline of oxidative stress and inflammation related diseases. Therefore, the phenolic compounds and flavonoids contained in medicinal plants have been intensely studied and their beneficial effects have been demonstrated in several animal studies. These compounds therefore contribute to the antioxidant power and act as anti-inflammatory agents (Talhouk et al., 2007). Viscum album known as mistletoe is a plant belonging to the family of Loranthacaea. This evergreen semi-parasitic shrub grows on various deciduous trees host and contains various biologically active substances (Shameem et al., 2018; Akbar, 2020). The main bioactive compounds in V. album are polysaccharides, phenylpropanes, lectins, viscositoxins, alkaloids, flavonoids, caffeic and other acids (Tahirović and Bašić, 2017).

Phytochemical composition depends on host tree and the antioxidant activity related to flavonoids and phenolic acids have been reported in several studies (Önay-Uçar et al., 2006; Papuc et al., 2010; Suk-Nam et al., 2016; Pietrzak et al., 2017; Trifunschi et al., 2017; Holandino et al., 2020). Different pharmacological effects of V. album have also been reported such as: in vitro anti-inflammatory activity of methanolic extract (Murthuza and Manjunatha, 2018), anti-hyperglycemnic and antioxidant activity of aqueous and ethanol extracts in STZ-diabetic rats (Orhan et al., 2005) antiepileptic, antipsychotic and sedative (Gupta et al., 2012), vasodilator and anti-hypertensive properties (Tenorio-Lopez et al., 2005; Ofem et al., 2007). Also, some studies cover anticancer effects of V. album samples (Siegle et al., 2001). In this study, we have quantified the phytoconstituent and evaluated the antioxidant and anti-inflammatory activities of V. album hosted by Persea americana which will help to provide novel insights into the discovery of new anti-inflammatory and antioxidant drugs and thus, develop effective treatment strategies for oxidative stress and inflammation-related disorders.

MATERIAL AND METHODS

Plant material

Fresh leaves of Viscum album were collected in Yaounde, Cameroon in the month of May 2017. The botanical identification was done at the National Herbarium of Yaounde (Cameroon) where voucher specimen was kept under the number 2974/HNC. The leaves of V. album collected were washed, dried in the shade and then crushed to obtain a fine powder.

Drugs and chemicals

Bovine serum albumin (BSA), casein, trypsin, diclofenac sodium, Folin-Ciocalteu, sodium carbonate, gallic acid, aluminium chloride, sodium acetate, rutin, vanillin, tannic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2’-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium ferricyanide, trichloroacetic acid, Iron III chloride, ascorbic acid, potassium persulfate, quercetin. All chemicals were purchased from Deagung, Korea and Sigma Aldrich St Louis, USA.

Preparation of plant extracts

Five hundred grams (500g) of powder were macerated respectively in 1 L of methanol, ethanol (70% ethanol-30% water) and water for 1 day. The macerate obtained was filtered and evaporated using a rotary evaporator (BUCHI Rotavapor R-300) at 50°C. After that, the filtrate was dried in an oven (Heraterm oven). The methanolic (MEV), hydroethanolic (HEV) and aqueous (AEV) extracts of V. album obtained were preserved in a refrigerator at 4°C for the quantification of phytochemical compounds, antioxidant activity and anti-inflammatory property. Decoction of V. album (DV) was separately prepared at 100°C during 20 min on a hot plate by mixing 10 g of powder with 250 mL of distilled water. The resultant mixture was filtered and the decoction obtained was dried in the oven. The extract obtained was kept for further analysis.

In vitro antioxidant activities

Estimation of total phenolic content

Total phenolic content was evaluated according to the spectrophotometric method using the Folin-Ciocalteu reagent described by Gao et al. (2000) with slight modifications. 0.2 mL of each extract and standard was put into a test tube, then 1.2 mL of distilled water and 0.2 mL of
Folin-Ciocalteu reagent were added. After standing for 3 min, 0.4 mL of sodium carbonate (7.5% Na₂CO₃) was added to the mixture. The tubes were homogenised with a vortex and incubated for 20 min in a 40°C water bath and the absorbance were read against a blank at 760 nm. Calibration curve was performed using a freshly prepared aqueous solution of gallic acid (0.05 mg/mL). The results were expressed in milligrams equivalent of gallic acid per gram of dry extract.

**Estimation of total flavonoid content**

The flavonoids content in the extracts was determined by the method described by Mimica-Dukic (1992). To 0.2 mL of each extract solution, 1.8 mL of distilled water and 1 mL of the aluminium chloride (AlCl₃) reagent were added. The AlCl₃ is consisted of 133 mg of aluminium chloride crystals and 400 mg of sodium acetate dissolved in 100 mL of the extraction solvent (methanol-distilled water-acetic acid 140:50:10; v/v/v). The extraction solvent was added and the whole solution was mixed with a vortex and the absorbance read against blank at 430 nm. The amount of flavonoids was calculated using a standard solution of rutin (0.1 mg/mL) and the results were expressed in milligrams of equivalent rutin per gram of dry extract.

**Estimation of tannin content**

Tannin was determined by using the spectrophotometric method using vanillin (Bainbridge et al., 1996). 1 mL of each extract was added to each test tube coated with aluminium foil to avoid light, followed by the addition of 3 mL of a freshly prepared solution of 4% vanillin in ethanol (w/v). After agitation, 1 mL of concentrated hydrochloric acid (HCl) was added to each tube and then incubated at room temperature for 15 min. The absorbance of the mixture was read at 500 nm against a blank. The amount of tannins was calculated using a standard calibration curve of tannic acid (0.1 mg/mL) and the results were expressed in milligrams of equivalent tannic acid per gram of dry extract.

**DPPH free radical scavenging test**

The antioxidant power which is the ability of a given substance to trap a free radical has been determined by the method described by Zhang and Hamauzu (2004) with some modifications. In a test tube containing 0.5 mL of different concentration (0.25 - 2.5 mg/mL) of each extract, 2 mL of DPPH (0.1 mM prepared in methanol) were introduced. Then, the mixture was stirred for 5 min and incubated in the dark for 60 min at room temperature. For the control tube, methanol was used in place of the extract. The reference used was ascorbic acid at different concentrations. Tests were repeated three times at each concentration level. Absorbance was read at 517 nm. The antioxidant activity was expressed as percent inhibition:

\[ I (%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \]

Where, Abs control is the absorbance of control tube and Abs sample is the absorbance of sample tube.

**ABTS radical scavenging activity**

It is one of the most widely used methods for the determination of the antioxidant activity of plant extract. The principle consists of following the kinetics of discoloration of the ABTS⁺ ion as described by Re et al. (1991). ABTS (2, 2'-azinobis-(3-ethylbenzothiazolin-6-sulphonic acid)) was prepared by mixing 0.0384 g of ABTS and 0.00662 g of potassium persulfate (K₅S₂O₈) with 10 mL of distilled water. The mixture was incubated for 16 h at room temperature, in the dark before use. For the actual analysis, the ABTS solution was diluted with ethanol and the absorbance adjusted to 0.700 (± 0.02) at 734 nm stable at 30°C (initial OD). In a test tube, 3.0 mL of this diluted ABTS solution were added in 30 μL of different extract of varying concentration (0.1 - 1 mg/mL). The tubes were agitated to homogenate the mixture. Absorbance reading was taken at 734 nm immediately after agitation. Ascorbic acid was used as an antioxidant reference at the same concentrations as the extracts. The percent inhibition was calculated according to the formula:

\[ I (%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \]

Where, Abs control is the absorbance of control tube and Abs sample is the absorbance of sample tube.

**Ferric-reducing antioxidant power (FRAP) assay**

The reducing power of iron (Fe³⁺) in the extracts was determined according to the method described by Oyaizu (1986). 1 mL of each extract was mixed with 2.5 mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of 1% potassium ferricyanide (K₃Fe(CN)₆) solution. The mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. To 2.5 mL of supernatant were added 2.5 mL of distilled water and 0.5 mL of an aqueous solution of iron chloride III (0.1% FeCl₃). The absorbance of the reaction mixture was read at 700 nm against a similarly prepared blank, by replacing the extract with distilled water, used to calibrate the apparatus (UV-VIS spectrophotometer). The positive control was represented by a solution of a standard
antioxidant; ascorbic acid whose absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested and the results were expressed in milligrams of ascorbic acid equivalent per gram of dry extract.

**In vitro anti-inflammatory assay**

**Protein denaturation inhibition assay**

*In vitro* anti-inflammatory activity of *Viscum album* was performed by using bovine serum albumin denaturation according to the method described by Padmanabhan and Jangle (2012) with some modifications. One mL of varying concentrations of the test extract or sodium diclofenac solution (100-500 µg/mL) was prepared in distilled water and 1 mL of 1% w/v bovine serum albumin was added to all the above test tubes. For control tests, 1 mL of distilled water instead of test solution was added. The test tubes were incubated at 27°C for 15 minutes and then heated at 70°C for 10 minutes. After cooling the tubes, the absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. Each experiment was done in triplicate. The inhibition of protein denaturation expressed in percentage was calculated using the following formula:

$$I \% = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of control tube and Abs sample is the absorbance of sample tube.

**Flavonoid content**

The flavonoid content in *V. album* extracts were expressed as milligrams equivalent rutin per gram of extract using the calibration curve of rutin (Figure 1b). The results presented in Table 1 showed that, methanolic and hydroethanolic extracts have higher flavonoid content respectively of 13.61 ± 0.01 mg RE/g and 15.94 ± 0.01 mg RE/g (p < 0.001). Lower flavonoid content was found in aqueous extract and decoction.

**Tannin content**

Table 1 presents the results of tannin content found in *V. album* extracts. These results are expressed as milligrams equivalent tannic acid per gram of extract using the calibration curve of the tannic acid (Figure 1c). Tannin content was found higher in methanolic and hydroethanolic extracts with 20.24 ± 0.04 mg TAE/g extract and 14.28 ± 0.04 mg TAE/g extract and 14.28 ± 0.04 mg TAE/g extract.
Figure 1: Standard calibration curve for the quantification of phytochemical compounds present in *Viscum album*. (a) standard curve of gallic acid for total phenolic content, (b) standard curve of rutin for flavonoid content, (c) standard curve of tannic acid for tannin quantification, (d) standard curve of ascorbic acid for ferric reducing antioxidant power.

Table 1: Total phenolic, flavonoid and tannin content of *Viscum album* extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenolic content (mg GAE/g extract)</th>
<th>Flavonoid content (mg RE/g extract)</th>
<th>Tannin content (mg TAE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>119.27 ± 0.01***</td>
<td>14.78 ± 0.01***</td>
<td>20.24 ± 0.04***</td>
</tr>
<tr>
<td>Hydroethanolic</td>
<td>124.56 ± 0.01***</td>
<td>17.11 ± 0.01***</td>
<td>14.28 ± 0.04***</td>
</tr>
<tr>
<td>Aqueous</td>
<td>45.29 ± 0.03</td>
<td>5.01 ± 0.01</td>
<td>3.11 ± 0.03</td>
</tr>
<tr>
<td>Decoction</td>
<td>25.52 ± 0.05</td>
<td>2.90 ± 0.02</td>
<td>3.88 ± 0.01</td>
</tr>
</tbody>
</table>

Each value is expressed in mean ± SD. ***p < 0.001 compared to aqueous and decoction extracts. One-way ANOVA followed by Tukey’s multiple comparison test. GAE: Gallic acid equivalent, RE: Rutin equivalent, TAE: Tannic acid equivalent, AAE: Ascorbic acid equivalent.

0.04 mg TAE/g extract respectively compared to aqueous extract with 3.11 ± 0.03 mg TAE/g extract and decoction with 3.88 ± 0.01 mg TAE/g extract (p < 0.001).

**DPPH free radical scavenging activity**

The antioxidant property of *V. album* leaves hosted by
**Table 2**: Inhibition percentage of DPPH radical by *Viscum album* extracts and SC$_{50}$ values.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Methanolic (%)</th>
<th>Hydroethanolic (%)</th>
<th>Aqueous (%)</th>
<th>Decoction (%)</th>
<th>Ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>70.21</td>
<td>66.80</td>
<td>68.09</td>
<td>68.58</td>
<td>98.71</td>
</tr>
<tr>
<td>1.25</td>
<td>68.49</td>
<td>64.29</td>
<td>54.76</td>
<td>61.08</td>
<td>97.19</td>
</tr>
<tr>
<td>0.625</td>
<td>65.24</td>
<td>55.52</td>
<td>42.99</td>
<td>55.46</td>
<td>86.57</td>
</tr>
<tr>
<td>0.25</td>
<td>60.58</td>
<td>52.28</td>
<td>33.33</td>
<td>42.92</td>
<td>83.93</td>
</tr>
<tr>
<td>SC$_{50}$ (mg/ml)</td>
<td>0.23***</td>
<td>0.205***</td>
<td>0.849</td>
<td>0.440</td>
<td>0.019***</td>
</tr>
</tbody>
</table>

SC$_{50}$: Scavenging concentration 50. Each value is presented as inhibition percentage of DPPH radical. ***p < 0.001 compared to aqueous extract and decoction. One-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 2**: Antioxidant activity of *Viscum album*: (a) Inhibition percentage of DPPH radical (b) Inhibition percentage of ABTS radical. MEV: Methanolic extract, HEV: Hydroethanolic extract, AEV: Aqueous extract, DV: Decoction. Each value is presented as mean ± SD (n = 3).

*Persea americana* was evaluated using DPPH radical scavenging. The results are presented on Table 2 and the representative graphic on Figure 2a. In general, these results showed that all tested extracts have exhibited good antioxidant activity on radical DPPH scavenging. The inhibition percentage increased gradually in a dose-concentration dependant manner (0.25 - 2.5 mg/mL). The lower concentration (0.25 mg/mL) of the aqueous extract and decoction showed an inhibition percentage less than 50%. The scavenging concentration 50 (SC$_{50}$) presented in Table 2 showed that, the higher antioxidant activity was found in methanolic extract with 0.23 mg/mL compared to aqueous and decoction extracts (p < 0.001). Lower activity was found in aqueous extract and decoction compared to the methanolic extract.

**ABTS$^+$ free radical scavenging activity**

Table 3 presents the results of the ABTS radical scavenging activity. Based on this study, it has been found that the different extracts from *V. album* have the ability of scavenging cation-radical ABTS$^+$. The highest level of scavenging radical was found in methanolic extract compared (p < 0.001) to the other extracts. The SC$_{50}$ was found lower in methanolic extract compared to the other extracts.

**FRAP analysis**

The results of the ferric reducing antioxidant power are presented on the Figure 3. The strongest ferric reducing ability was found in hydroethanolic extract (785.32 ± 0.03 mg AAE/g) followed by methanolic extract (766.52 ± 0.02 mg AAE/g). The lowest activity was obtained with aqueous extract (124.19 ± 0.01 mg AAE/g) and decoction (187.99 ± 0.03 mg AAE/g).

**Anti-inflammatory activity**

**Inhibition of protein denaturation**

The extracts of *V. album* were analysed for their anti-
Table 3: Inhibition percentage of ABTS radical of Viscum album and SC50 values.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Methanolic (%)</th>
<th>Hydroethanolic (%)</th>
<th>Aqueous (%)</th>
<th>Decoction (%)</th>
<th>Ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90.28</td>
<td>80.18</td>
<td>54.62</td>
<td>63.82</td>
<td>94.07</td>
</tr>
<tr>
<td>0.5</td>
<td>77.34</td>
<td>61.26</td>
<td>43.81</td>
<td>45.99</td>
<td>91.99</td>
</tr>
<tr>
<td>0.25</td>
<td>54.67</td>
<td>47.42</td>
<td>39.45</td>
<td>32.43</td>
<td>84.59</td>
</tr>
<tr>
<td>0.1</td>
<td>42.15</td>
<td>36.37</td>
<td>26.79</td>
<td>27.41</td>
<td>80.42</td>
</tr>
<tr>
<td>SC50 (mg/mL)</td>
<td>0.159***</td>
<td>0.237***</td>
<td>0.713</td>
<td>0.543</td>
<td>0.008***</td>
</tr>
</tbody>
</table>

SC50: Scavenging concentration 50. Each value is presented as inhibition percentage of ABTS radical. ***p < 0.001 compared to aqueous extract and decoction. One-way ANOVA followed by Tukey’s multiple comparison test.

Figure 3: Ferric reducing antioxidant power (FRAP) of Viscum album extracts. MEV: Methanolic extract, HEV: Hydroethanolic extract, AEV: Aqueous extract, DV: Decoction. Each value is presented as mean ± SD (n=3).

Table 4: Inhibition percentage of protein denaturation of Viscum album extracts and IC50 values.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Methanolic (%)</th>
<th>Hydroethanolic (%)</th>
<th>Aqueous (%)</th>
<th>Decoction (%)</th>
<th>Diclofenac sodium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>90.05</td>
<td>87.46</td>
<td>86.25</td>
<td>87.97</td>
<td>88.62</td>
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<tr>
<td>300</td>
<td>83.02</td>
<td>81.83</td>
<td>72.97</td>
<td>67.39</td>
<td>83.54</td>
</tr>
<tr>
<td>200</td>
<td>74.77</td>
<td>75.54</td>
<td>62.16</td>
<td>60.84</td>
<td>80.05</td>
</tr>
<tr>
<td>100</td>
<td>68.72</td>
<td>65.36</td>
<td>58.72</td>
<td>54.77</td>
<td>76.90</td>
</tr>
<tr>
<td>IC50 (µg/ml)</td>
<td>40.70***</td>
<td>43.89***</td>
<td>79.85</td>
<td>106.65</td>
<td>8.25***</td>
</tr>
</tbody>
</table>

IC50: Inhibition concentration 50. Each value is presented as inhibition percentage of protein denaturation. ***p < 0.001 compared to aqueous extract and decoction. One-way ANOVA followed by Tukey’s multiple comparison test.

inflammatory activity and compared with the diclofenac sodium standard. The results presented in Table 4 showed that, the inhibition percentage of all the extracts increased with concentration (100-500 µg/ml) compared as well as for the diclofenac sodium. The inhibitory concentration 50 (IC50) of diclofenac was found at 8.25 µg/ml. Methanolic and hydroethanolic extracts significantly (p < 0.001) have a higher inhibitory concentration of protein denaturation in regard of the IC50 which were 40.70 µg/ml and 43.89 µg/ml respectively compared to aqueous extract and decoction.

Proteinase inhibitory activity

All extracts exhibited significant anti-proteinase activity at different concentrations as presented in Table 5. At the concentration of 100 µg/ml, aqueous extract and decoction showed an inhibition percentage lower than 50% compared to methanolic and hydroethanolic extracts. Diclofenac sodium showed the maximum inhibition 87.65% at 1000 µg/ml. Based on the IC50, aqueous extract and decoction have shown lower proteinase inhibitory activity.
respectively of 157.63 µg/ml and 135.58 µg/ml compared to methanolic and hydroethanolic extracts where the proteinase inhibition activity were significantly high (p < 0.001).

**DISCUSSION**

Oxidative stress and inflammation are the two predominant driving forces behind various metabolic and degenerative diseases. Thus, prevention of oxidative insults and chronic inflammation is the primary step for the prevention of these disorders. The present study analysed the antioxidant and anti-inflammatory properties of *Viscum album* extracts hosted by *Persea americana*. Medicinal plants have always been considered as a healthy source of life worldwide. Therapeutic properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants rely on their 100% natural property (Hossain et al., 2012). They have been recognized as important sources of therapeutically active compounds for so long. These active compounds have been linked to secondary metabolites such as phenolic compounds (Adebayo et al., 2015). Phenolic compounds are ubiquitous secondary metabolites in plants which possess a wide range of therapeutic uses such as antioxidant and anti-inflammatory activities, free radical scavenging activities and also decrease cardiovascular complications (Anoop et al., 2015; Yadang et al., 2019).

The results of this study have shown that, the extracts of *V. album* possess a high amount of total phenolics, flavonoids and tannins content. Results of previous studies (Papuc et al., 2010; Pietrzak et al., 2017; Trifunski et al., 2017) demonstrated also that, *V. album* presented highest amount of polyphenols and flavonoids in different extracts of mixed polar organic solvent with water. Holandino et al. (2020) observed an increase in flavonoid content of *V. album* ethanolic extract. Also, Suk-Nam et al. (2016) and Tahirović and Bašić (2017) reported a high amount of phenolic compounds and flavonoids in hydroethanolic and methanolic extracts of *V. album* respectively harvested in different host trees. From all these previous results, we can affirm that *V. album* exhibited high amount of phenolic compounds depending of the host trees. It is reported that phenolic compounds and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms (Shirwaike et al., 2004). Thus, plants containing phenolic compounds such as flavonoids, tannins, and phenols have been reported to act like scavengers of free radicals, reactive oxygen species (ROS) and they also chelate metal ions (Hussain et al., 2016). Several previous studies have reported the antioxidant capacity of some *Viscum* species hosted in different trees. Different methods have been used for the determination of antioxidant activity of plants. In this study, three methods were used to assess the antioxidant activity of *Viscum album*.

The DPPH radical scavenging analysis is used to measure the electron donating ability of the plants. DPPH is a stable radical commonly used to determine the antioxidant activity of various compounds (Amoussa et al., 2015). This method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant. The results of this study revealed that, at lower concentrations, methanolic and hydroethanolic extracts exhibited a percentage of DPPH inhibition higher than 50% compare to aqueous and decoction extracts. Results are consistent with previous studies (Onay-Uçar et al., 2006; Shameem et al., 2018) where the methanolic extract of *V. album* hosted in different trees presented high DPPH scavenging radical showing a high antioxidant activity due to the phenolic compounds it contains. Also, the results of Papuc et al. (2010) showed that, the ethanolic crude extract of *V. album* exhibited high DPPH scavenging radical, reducing power and ion chelating capacity. The SC50 of the methanolic extract is lower compared to the other extracts followed by hydroethanolic extract. The lower the SC50 value, the stronger the antioxidant power; meaning that, the methanolic and hydroethanolic extracts are better DPPH radical scavengers than the aqueous extracts. ABTS is the most widely used methods for determining the antioxidant activity of plant extracts. The test measures the antioxidants’ capacity to neutralise the 2, 2’-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) (ABTS•+) stable radical cation, a blue-green chromophore of maximum absorption at 734 nm, whose intensity decreases in the presence of

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Methanolic (%)</th>
<th>Hydroethanolic (%)</th>
<th>Aqueous (%)</th>
<th>Decoction (%)</th>
<th>Diclofenac sodium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>80.742</td>
<td>79.015</td>
<td>83.813</td>
<td>84.837</td>
<td>87.652</td>
</tr>
<tr>
<td>500</td>
<td>73.832</td>
<td>75.048</td>
<td>72.086</td>
<td>74.453</td>
<td>82.022</td>
</tr>
<tr>
<td>250</td>
<td>65.195</td>
<td>64.127</td>
<td>56.711</td>
<td>52.981</td>
<td>77.863</td>
</tr>
<tr>
<td>100</td>
<td>58.049</td>
<td>52.627</td>
<td>42.425</td>
<td>48.951</td>
<td>68.522</td>
</tr>
<tr>
<td>IC50 (µg/ml)</td>
<td>54.87 ***</td>
<td>80.80 ***</td>
<td>157.63</td>
<td>135.58</td>
<td>18.25 ***</td>
</tr>
</tbody>
</table>

IC50: Inhibition concentration 50. Each value is presented as inhibition percentage of proteinase activity. ***p < 0.001 compared to aqueous extract and decoction. One-way ANOVA followed by Tukey’s multiple comparison test.
antioxidants (Munteanu and Apetrei, 2021). Thus, antioxidant capacity is measured as the ability of extract to decrease the colour reacting directly with the ABTS radical. The results of this study showed that, at the concentration of 1 mg/mL, the percentage of ABTS inhibition was higher in methanolic and hydroethanolic extracts compared to aqueous and decoction.

Previous study of Rahmawati et al. (2014) has demonstrated that, all the V. album extracts of different host trees have the ability of scavenging cation-radicals ABTS+. The methanolic extract of V. album harvested from different host trees has shown high antioxidant activity (Tahirović and Bašić, 2017). The study results of Vicaș et al. (2008, 2011) showed that, aqueous and ethanol leaf extracts from three different seasons have the ability of scavenging cation-radicals ABTS+ showing a highest level of scavenging radicals. These results are in concordance with other investigators who found that, antioxidant capacity differs depending on the host trees (İnay-Uçar et al., 2006). The lower the SC50 value, the greater the antioxidant activity. The SC50 value of methanolic and hydroethanolic extracts of V. album in this study were 0.159 µg/mL and 0.237 µg/mL respectively which describe better free radical scavenging ability of this plant.

Indeed, the IC50 of methanolic and hydroethanolic extracts were observed lower compared to the aqueous extract and decoction. Didofenac was used as a standard anti-inflammatory drug. Thus, any phytocomponent which has the ability to inhibit the denaturation of proteins could contribute significantly in stopping the course of inflammation. Neutrophils are known to be a rich source of serine proteinase and are localized in lysosomes. It was previously reported that, leukocytes proteinase plays an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995; Leelaprakash and Mohan, 2011). This investigation showed that, all the extracts possess significant anti-proteinase activity. Therefore, the anti-inflammatory activity of the tested samples may also be due to the anti-proteinase activity of the extracts. The study results of Murthuza and Manjunatha (2018) have also shown that, V. album exhibited significant anti-proteinase activity. The maximum inhibition percentage was observed in V. album methanol extract (59.91 ± 1.90). Among all the extracts, methanolic and hydroethanolic extracts of V. album showed pronounced antioxidant activity and good inhibitory activity against protein denaturation and proteinase. Therefore, the capacity of Viscum album to scavenge free radicals and to inhibit protein denaturation and proteinase activity could be responsible of its ability to slow down the progression of metabolic and neurodegenerative disorders.

CONCLUSION

The antioxidant and the anti-inflammatory activities of Viscum album hosted by Persea americana were evaluated in this study. The different extracts were found to possess
antioxidant properties and anti-inflammatory activities. The phytochemical composition showed that the extracts have a large amount of total phenolic compounds, flavonoids and tannins. Among the different extracts, the methanolic and the hydroethanolic extracts have shown the highest DPPH and ABTS radical scavenging activities compare to the aqueous extract and decoction. *In vitro* anti-inflammatory studies of *V. album* extracts exhibited the protein denaturation inhibition and proteinase inhibition. From all the results above, *Viscum album* could be a promising source of antioxidant with a good anti-inflammatory activity which can be used to remedy to oxidative stress and inflammation related diseases.

REFERENCES


