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1 **Antioxidant properties, protein binding capacity and mineral contents of some plants**
2 **traditionally used in the management of animal wounds**

3

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19

20 **Abstract**

21 Herbal medicines are considered an intricate and integral part of humankind's knowledge
22 systems. Time has proven their efficacy and safety for both human and animal applications.
23 Modern science, guided by indigenous knowledge systems can further optimize the use of various
24 herbal products. To widen the current focus on herbal medicines, a study was carried-out to
25 determine antioxidant properties, phytochemical and mineral contents of some medicinal plants
26 used in ethnoveterinary practices in the management of animal wounds in Zimbabwe. The studied
27 plants were *Cissus quadrangularis* L, *Erythrina abyssinica* Lam. ExDC. and *Adenium multiflorum*
28 Klotzsch). Radical scavenging activities, antioxidant properties were determined using the DPPH
29 and the *β*-carotene-linoleic acid model while the total phenolic content was determined using the

Folin C method, flavonoid content using the aluminium assay and mineral content was investigated using the ICP-OES method. All extracts investigated exhibited radical scavenging activities and antioxidant properties, with *C. quadrangularis* leaf extracts exhibiting superior activities such as radical scavenging (EC_{50} of 21.04 ± 3.00 µg/ml) and antioxidant properties (ORR of 0.03 ± 0.01). Variations were observed in the total phenolic, flavonoid and metal contents. *C. quadrangularis* leaf extracts exhibited highest amounts of total phenolic and flavonoid contents. The *E. abyssinica* (bark) and *A. multiflorum* extracts exhibited moderate (40–70%) affinity for protein binding while the rest of the extracts exhibited high affinity. Their antioxidant properties, phytochemical profile and mineral content justify applications in animal wound management and many other human and/or animal uses.

Key words: Antioxidants; Free radicals; Phenolic compounds; Skin infections; Wound healing

1. Introduction

Humankind and their communities have benefited, to a large extent, from their indigenous knowledge systems for innumerable years. Such knowledge systems include plants useful for animal and/or human disease management. Several authors have identified many advantages of using herbal medicines including low costs, accessibility, high potency, good tolerance and few side effects among others (Pathak and Das, 2013). Several adverse side effects coupled with exorbitant costs of modern pharmaceutical medicines have contributed to the growing use of herbal medicines in the management of both human and animal diseases. Zimbabwe has a very large untapped botanical wealth. It has many similarities with her southern neighbour South Africa which has around 24 000 species comprising more than 10% of the world's vascular plant flora (Germishuizen and Meyer, 2003).

The animal skin and associated membranes makes the largest organ and the first-line of defence. The skin is constantly exposed to infectious organisms and other toxicants (Mishra, 2011). Soyelu and Masika (2009) have defined animal wounds in a manner that includes sores,

abscesses, warts and inflamed skin lesions.These forms of tissue damage have several outcomes such as acute inflammation (representing initial response aimed at eliminating dead material and infection minimisation), restitution (i.e. damaged tissue replaced by identical tissue which is ideal), fibrous repair (scar tissue) and/or chronic inflammation (persistent tissue damage). Wounds such as lesions, warts, sores or abscesses may also be complicated with pathogenic microorganisms like bacteria (e.g. *Staphylococcus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia plymuthica*, *Proteus mirabilis*, *Salmonella* species); fungi (e.g. *Candida albicans*, *Trichophyton mentagrophytes* var. interdigitale, *Aspergillus* species, *Fusarium* species) and even viruses like *Papillomavirus* (Becker et al. 1991; Crutchfield et al. 2005; Alghalibi et al. 2011; Shakoor et al. 2012).

Plants produce a diverse array of secondary metabolites with many functions, such as defence against microbial and viral invasions. Such molecules have the ability to inhibit growth of infectious microorganisms, offer good antioxidant and anti-inflammatory properties, and provide rare micro-nutrients and minerals. All the listed molecules possesses wound healing and restitution properties. *Dalbergia nyasae* Bak. f., *Cissus quadrangularis* L., *Adhatoda vasica* Nees, *Annona squamosa* L., *Helianthus annuus* L., *Curcuma amada* Roxb., *Hypericum hookerianum* Wight & Arn., *Sida cordifolia* Linn., *Semecarpus anacardium* Linn., *Coelogyne cristata,* among others are known to help in soft, nervous, bone and muscle tissue repair among other healing powers (Deka et al. 1994; Shah, 2011; Jaiswal et al. 2004). However, there is a limitation of information on the validation of these species in ethnoveterinary studies. Three plant species used in traditional animal and human wound management were selected in the study based on an ethnopharmacological study conducted in some areas of Zimbabwe. Table 1 summarises the traditional human and ethnovetarinary uses of the three plants namely: *Cissus quadrangularis* L., *Adenium multiflorum* Klotzsch and *Erythrina abyssinica* Lam. Ex DC.

The current study was conducted to determine the antioxidant properties, phytochemical and mineral profiles of these three plant species commonly used in the management of animal wounds in Zimbabwe.

2. Materials and methods

2.1 Plant collection and identification

87 The plant materials were collected from Mberengwa, Midlands Province (*C. quadrangularis*–20°28'09.0"S 29°55'23.3"E.), Karoi, Mashonaland West Province (*E. abyssinica*– 16°49'44.1"S 29°41'19.8"E) and Buhera, Manicaland Province *(A. multiflorum* – 19°17'10.7"S 31°25'20.2"E) of Zimbabwe during the months of October – December 2014. Species identification was done by qualified botanists from the National Herbarium and Botanic Garden and University of Zimbabwe, Harare, Zimbabwe where specimens were submitted.

93

94 **2.2 Preparation and extraction**

95 Fresh leaf and stem samples from *C. quadrangularis,* whole plant of *A. multiflorum* and leaf, bark samples of *E. abyssinica* were separately oven dried at 50°C for 48h. Dried plant materials were ground into powders and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for 1h. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The extracts were then concentrated under pressure using a rotary evaporator at 30°C and completely dried under a stream of air. Fresh extracts of 50% aqueous methanol were used in the phytochemical analysis while the dried ones were dissolved in 50% methanol to determine concentrations for the antioxidants assays.

103

104 **2.3 Bioassays: Antioxidant activity and phytochemical levels**

105 *2.3.1DPPH radical scavenging activity*

106 The DPPH radical scavenging assay was done as described by Karioti *et al*. (2004), with relevant modifications. Briefly, 15 µL of each plant extract diluted with methanol were added to a methanolic DPPH solution to give a final volume of 1.5 ml. The concentration of DPPH in the resultant final reaction was 50 µM. The reaction mixtures were prepared under dim light conditions and incubated at room temperature for 30min. The decreases in the purple colouration of the reaction mixtures were read using a spectrophotometer at 517 nm. Standard antioxidant i.e. ascorbic acid (e.g. 5, 10, 20, 40, 80 µM) solutions were used as positive controls. Solutions with the same chemicals except for the extracts or standard antioxidants were used as negative controls. Methanol was used to blank the spectrophotometer. The background correction was done by

115 subtracting the absorbance of the extracts without DPPH. Each test was done in triplicate. The free
116 radical scavenging activity (RSA) as determined by the decolouration of the DPPH solution was
117 calculated according to the formula;

118 $$\text{RSA (\%)} = \left\{1 - \left(\frac{\text{Abs}_{517\text{ nm}}\ \text{Sample}}{\text{Abs}_{517\text{ nm}}\ \text{Neg Control}}\right)\right\} \times 100,$$

119 where Abs_{517} sample is the absorbance of the reaction mixture containing the extract or positive
120 control solution and Abs_{517}Neg control is the absorbance of the negative control (Karioti *et al.*
121 2004). Radical scavenging activity (%) was plotted against the extract concentration. The EC_{50}
122 values, representing the amount of extract required to decrease the absorbance of DPPH by 50%
123 were calculated from the logarithmic non-linear regression curve.

124

125 *2.3.2 β-Carotene-linoleic acid model system (CLAMS)*

126 The delay or inhibition of *β*-carotene and linoleic acid oxidation was measured according
127 to the method described by Amarowicz *et al.* (2003) and Ndhlala *et al.* (2014) with minor
128 modifications. *β*-carotene (10 mg) was dissolved in 10 ml chloroform in a brown Schott bottle.
129 The excess chloroform was evaporated under vacuum, leaving a thin film of *β*-carotene. Linoleic
130 acid (200 µl) and Tween 20 (2 ml) were immediately added to the thin film of *β*-carotene and
131 mixed with aerated distilled water (497.8 ml), giving a final *β*-carotene concentration of 20 µg/ml.
132 The mixture was further saturated with oxygen by vigorous agitation to form an orange coloured
133 emulsion. The emulsion (4.8 ml) was dispensed into test tubes to which 200µl of the resuspended
134 plant extracts at 6.25 mg/ml or butylated hydroxytoulene (BHT) (6.2 5mg/ml) were added, giving
135 a final concentration of 250 µg/ml in the reaction mixtures. Absorbance for each reaction was
136 immediately ($t = 0$) measured at 470 nm and incubated at 50°C, with absorbance of each reaction
137 mixture being measured every 30 min for 180 min. Tween 20 solution was used to blank the
138 spectrophotometer. The negative control consisted of 50% methanol in place of the sample. The
139 rate of *β*-carotene bleaching was calculated using the following formula;

140 $$\text{Rate of bleaching (R)} = \left\{\ln\left(\frac{A_{t=0}}{A_{t=t}}\right)\right\} \times \frac{1}{t}$$

141 where $A_{t=0}$ is the absorbance of the emulsion at 0 min; and $A_{t=t}$ is the absorbance at time t. The
142 calculated average rates were used to determine the antioxidant activity (ANT) of the respective
143 herbal preparations, and expressed as percent inhibition of the rate of β-carotene bleaching using
144 the formula;

145 $$\% \text{ ANT} = \left(\frac{R_{control} - R_{sample}}{R_{control}}\right) \times 100$$

146 where $R_{control}$ and R_{sample} represent the respective average β-carotene bleaching rates for the control
147 and plant extract, respectively. Antioxidant activity was further expressed as the oxidation rate
148 ratio (ORR) based on the equation;

149 $$\text{ORR} = \frac{R_{sample}}{R_{control}}$$

150 *2.3.3 Total phenolic assay*

151 The total phenolic assay was based on Makkar, (1999) making necessary modifications.
152 Aliquots (0.05 ml) of the extracts were placed in test tubes with the volumes adjusted to 1ml with
153 distilled water. To the resultant solutions 500 µl of the Folin-Ciocalteu reagent were added
154 followed by 2.5 ml of the sodium carbonate solution. The resultant solutions were read at 725 nm
155 after 40 min. The amounts of total phenols were calculated as tannic acid equivalents from a
156 standard curve and expressed as a percentage.

157

158 *2.3.4 The total flavonoid assay*

159 Aliquots (50 µl) of each extract adjusted to 1ml by absolute methanol were mixed with
160 methanol–HCl (2.5 ml) and vanillin reagent (2.5 ml) before incubation for 20 minutes at 30ºC.
161 Absorbance of those resultant reaction mixtures were read at 500 nm. A reaction mixture
162 containing methanol instead of plant extracts was used as the negative control. Determination of
163 the flavonoids was done in triplicates, and the concentration was expressed as catechin equivalents
164 per g of extract based on a standard curve (Ndhlala et al. 2008; Muchuweti etl al.2005).

165

166 **2.4 Bioassays: Determination of protein-precipitating capacity of phenolic compounds as a**
167 **model for wound healing**

168 The determination of the protein-precipitating capacity of the phenolics in the 50% aqueous
169 methanol extracts was done according to Makkar (1999) as outlined below.

170 *2.4.1 Formation of the phenolics-protein complex*

171 To 2 ml of bovine serum albumin (BSA) solution (containing 1 mg BSA/ml acetate
172 buffer), 50 % aqueous methanol was added to the 50 % aqueous methanol extract to make 3 ml (in
173 increasing concentration of 50 % aqueous methanol extract vs 50% aqueous methanol as follows:
174 0.95, 0.90, 0.85, 0.80, 0.75, 0.70 ml of 50 % methanol with 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 ml of
175 the MeOH extract), in triplicate in a centrifuge tube. The contents were then mixed on a vortex
176 machine and allowed to stand at 4 °C overnight in a refrigerated centrifuge. The following day (16
177 h), the tubes were centrifuged at 1370 × *g* for 10 min. The supernatant was carefully removed
178 without disturbing the precipitate. To the precipitate, 1.5 ml of 1 % sodium dodecyl sulfate (SDS)
179 solution was added and mixed on a vortex machine until it dissolved. The resultant solution
180 contained the dissolved phenolics-protein complex.

181

182 *2.4.2 Determination of phenolics in the phenolics-protein complex*

183 Aliquots (1 ml) of the above dissolved phenolics-protein complex were transferred
184 into clean sets of test tubes. To the tubes, 3 ml of SDS-triethanolamine solution (1 % SDS (w/v)
185 and 7 % (v/v) triethanolamine in distilled water) were added, followed by 1 ml ferric chloride
186 reagent (0.01M ferric chloride in 0.1 M HCl). Absorbance readings were taken at 510 nm after 30
187 min of incubation at room temperature using a UV-visible spectrophotometer. The absorbance
188 readings were converted to gallic acid equivalents, using a standard curve. The obtained
189 equivalents were multiplied by 1.5 to obtain the phenolics in the complex. A linear regression
190 curve between phenolics precipitated as gallic acid equivalents and mg dry plant samples (in
191 aliquot taken for the assay) was plotted using GraphPad Prism V6 (GraphPad Prism® software
192 Inc. CA). The slope of the curve (mg phenolics precipitated/mg plant samples = x) represented the
193 protein-precipitating phenolics in the sample (Makkar, 1999).

194

195 *2.4.3 Protein-precipitating capacity as a percentage of total phenolics*

196 Different aliquots (50 to 550 ml) of the 50 % aqueous methanol extracts were made
197 up to 1 ml with 1 % of SDS, and 3 ml of the SDS-triethanolamine solution were added, followed
198 by 1ml of ferric chloride reagent. After incubation at room temperature for 30 min, absorbance at
199 510 nm was obtained as described above. A linear regression curve between phenolic acid
200 equivalents and mg sample (in the aliquot taken) was drawn using GraphPad Prism software. The
201 slope of the curve (mg phenolics equivalent/mg sample = y) represented the total phenolics. The
202 protein-precipitating phenolics have already been measured as x (Section 2.5.4 above).

203 The percentage of total phenolics which can precipitate protein = $(x / y) \times 100$.

204

205 *2.4.4. Statistical analysis*

206 Data from phenolic analysis and protein-precipitating capacity of samples collected
207 between the two locations were compared using Student t-test and the level of significance set at
208 $P < 0.05$.

209

210 **2.5 Elemental analysis**

211 After adequate acid and catalyst based digestion, elemental analysis for most of the
212 elements were done using Inductively Coupled Plasma-Optical Emission Spectrophotometry ICP-
213 OES (Varian 720-ES, Varian Inc, Palo Alto, CA, USA). ICP-OES provides for a multi-elemental
214 analysis and supports broad linear calibration range. For phosphorus the samples were analysed
215 on a Segmented flow Analyser (Seal AA3). This automated procedure is based on colorimetric
216 method in which a yellow colour is formed by the reaction of phosphate with molybdovanadate in
217 an acidic medium. The phosphor-molybdovanadate complex was read at 420nm.Nitrogen
218 determination was done based on the blue compound formed by reacting the sample with salicylate
219 and sodium hypochlorite solution read at 660 nm. Nitroprusside was used as a catalyst.

220

221 **3. Results and discussion**

222 *Cissus quadrangularis* has many applications in traditional medicines. Chief among its
223 uses is the management of various diseases including osteoporosis, septic wounds, bone fractures,
224 asthma, cough, haemorrhoids, gonorrhoea, epistaxis, scurvy, irregular menstruation among other
225 uses (Marandure, 2016; Gulzar et al. 2015; Raj and Joseph, 2011). *Adenium multiflorum* has been
226 used to manage warts and ectoparasites by small-holder farmers for years in Zimbabwe
227 (Marandure, 2016). The plant and others in its genus are rather most known for their potential toxic
228 effects mediated by digestive disturbances and cardiac insufficiency (Burrows and Tyrl, 2013).
229 *Erythrina abyssinica* widely distributed in Zimbabwe, Tanzania, Eritrea, Ethiopia, Kenya and
230 Uganda also has wider human and animal nutritional and/or medicinal applications (Temesgen
231 and Tamir, 2015).

232 All the plant extracts particularly those of *C. quadrangularis* leaves, *E. abyssinica* bark
233 and *A. multiflorum* showed high radical scavenging and antioxidant properties as presented in
234 Table 2. Leaf extracts of *C. quadrangularis* exhibited the highest radical scavenging (EC_{50} of 21.04
235 ± 3.00[a] µg/ml) and antioxidant properties (ORR of 0.03 ± 0.01) compared to the other plant species
236 studied. Other *in vivo* studies have shown that *C. quadrangularis* extracts can inhibit lipid
237 peroxidation, free radical formation and enhance the activities of antioxidant enzymes (Raj and
238 Joseph, 2011). Thus the demonstrated free radical scavenging and antioxidant properties can
239 explain the use of *C. quadrangularis* in wound management where it prevents further tissue
240 damage and facilitate repair and healing. Free radical scavengers and antioxidants discourage the
241 proliferation of pathogenic microbes around the wounded area.

242 Phenolic and flavonoid compounds are essential plant secondary metabolites, with several
243 key functions in plant metabolism such as plant-microorganism communications, stimulants,
244 pigments, flavourants and protection molecules (Kumar and Pandey, 2013; Amjad and Shafighi,
245 2013; Ghasemzadeh and Ghasemzadeh, 2011). Phenolic and flavonoid compounds have
246 significant biologic activities that are key in wound healing such as radical scavenging/anti-
247 oxidative, anti-allergic/anti-inflammatory, antiviral, antibacterial, antifungal, hypoglycaemic and
248 anti-carcinogenic properties/effects (Kumar and Pandey, 2013, Amjad and Shafighi, 2013;

249 Agrawal, 2011; Stanković, 2011). The study demonstrated the presence of both phenolic
250 compounds/tannins and flavonoids in all the plant extracts as presented in Figure 1 and 2). *C.*
251 *quadrangularis* leaf extracts exhibited highest levels of total phenolic compounds (more than 20
252 GAE/g) further explaining its wider applications in ethnomedicine as these compounds have
253 several pharmacological activities. Mohanambal et al. (2011) despite demonstrating the presence
254 of many other phytoconstituents failed to detect alkaloids and flavonoids in *C. quadrangularis*
255 extracts. A study done by Gulzar et al. (2015) also failed to show the presence of tannins and other
256 phytoconstituents. Their findings are in contrast with the findings of this and other studies (Shabi
257 Ruskin et al. 2014). These noted differences in findings demonstrate the complexity of
258 phytochemical studies. Variations of phytoconstituents can arise from differences in geographical
259 regions, seasons, methods of sample collection, storage, preparation, extraction methods among
260 other reasons. *Erythrina abyssinica* as in this study was also reported to contain moderate amounts
261 of phenolic compounds/tannins and relatively high flavonoids by Mariita et al. (2010). Several
262 alkaloids and flavonoids have been isolated from several plants of the genus *Erythrina* (de Araújo-
263 Júnior et al. 2012). Phytochemical profile of *E. abyssinica* can adequately explain its wide
264 applications in ethnomedicine.

265 The results of the different essential elements and micro-nutrients in all the plant species
266 studied are presented in Table 3. Nutrients maintain and support all phases of wound healing
267 (Demling, 2009). For proper wound healing to occur there is need for mobilization of various
268 immune cells and other necessary nutrients such as amino acids, vitamins and trace elements
269 (Berger et al. 2007). Zinc is a trace element that has a significant role in cellular growth and
270 replication, rapid epithelialization (cofactor in collagen synthesis and other key proteins), cellular
271 immunity, wound healing and protection against oxidative stress (Agarwal et al. 2011; Demling,
272 2009; Berger et al. 2007). Copper is essential for wound healing (cofactor in connective tissue
273 production and cross-linking of collagen), haematopoiesis and as an antioxidant (Agarwal et al.
274 2011; Demling, 2009; Berger et al. 2007). Other trace elements selenium, iron, manganese, are
275 also key in different stages of the wound healing process such as defence against oxidative
276 stresses, carbohydrate and lipid metabolism, oxygen transport and various as co-enzymes/co-
277 factors (Agarwal et al. 2011; Demling, 2009). The results obtained in this study are in agreement
278 with studies by Raj and Joseph (2011), Sen and Dash (2012) who also demonstrated significant
279 presents of many of these microelements. The presence of these elements can also explain several

280 pharmacological and nutritional capabilities of *C. quadrangularis*, *E. abyssinica* and *A.*
281 *multiflorum*, especially in wound healing.

282 The results for the protein-binding capacity of the 50% methanolic extracts of the plant
283 species studied are presented in Table 4. Four levels of affinity were described, with 0-20% being
284 considered insignificant, 20–40% low, 40–70% moderate, and 70–100% high (Ndhlala et al 2014).
285 The *E. abyssinica* (bark) and *A. multiflorum* extracts exhibited moderate affinity for protein
286 binding while the rest of the extracts exhibited high affinity. Wound healing is a complex is a
287 systematic process, which was traditionally explained in terms of 4 overlapping classic phases:
288 hemostasis, inflammation, proliferation, and maturation but recently, overlapping stages has been
289 explained that could include exclusion of bacterial and fungal infections as well as re-
290 epithelialization, extracellular matrix (ECM) formation and remodeling (Perini et al., 2015).

291 Phenolic-protein complexes form a film which limits fluid loss and forms a physical
292 barrier to microbial infections and forms insulations on damaged tissue protecting the wound from
293 environmental harm (Luseba et al., 2007). Phenolic compounds can therefore be involved at any
294 stage as is described of wound healing including the hemostasis, inflammation, proliferation, and
295 maturation as well as the other processes such as exclusion of bacterial and fungal infections as
296 well as re-epithelialization, extracellular matrix (ECM) formation and remodeling (Bruneton,
297 1995).

298 The hydroxyl group in phenolic compounds exhibits high protein affinity resulting
299 in the above mentioned films which forms the physical barriers that aid wound healing. On
300 microbial exclusion, the extracts with high protein binding affinity could exert their effects by
301 forming hydrophobic and hydrogen bonding with the protein regions of the bacterial cell wall.
302 High protein binding affinity has in some cases pronounced a nonsense as they could transform
303 into major negative impact on human and animal nutrition. High protein binding phenolic
304 compounds, especially those rich in tannins influence protein utilisation within the body and is
305 generally viewed adversely by reducing the bioavailability of nutrients and medicinal value of
306 plants.

307

308 **Conclusions**

309 The phytochemistry and element content of *C. quadrangularis*, *E. abyssinica* and *A.*
310 *multiflorum* extracts justifies their use in wound management and many other ethnomedicinal
311 applications. *C. quadrangularis* exhibited the highest total phenolics, flavonoids and antioxidant
312 properties as compared to the other plant species studied here. There is need for both in vitro and
313 in vivo pharmacological and toxicological evaluations of *Cissus quadrangularis*, *Erythrina*
314 *abyssinica* and *Adenium multiflorum* extracts. These evaluations coupled with literature reviews,
315 purity and formulation studies may result in the development of effective medicinal products for
316 animal and human use.

317

318 **Conflict of interest**

319 No conflicts of interest to declare.

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Table 1

Family name	Scientific name	Common name	Traditional uses	Ref
Apocynaceae	*Adenium multiflorum* Klotzsch	Impala Lily- Eng; *Chisvosve* - Shn	Used to treat warts, calluses and other hard inflamed areas of the skin	Van Wyk and Gericke, 2000.
Fabaceae	*Erythrina abyssinica* Lam. Ex DC.	Uganda coral, erythrina, flame tree, lucky bean tree – Eng; Munhimbiti, Mutiti - Shn	Used in the management of topical inflammation and infections especially involving mucus membranes as in eyes	Orwa et al., 2009
Vitaceae	*Cissus quadrangularis* L.	veldt grape or devil's backbone - Eng; *Muvengahonye* - Shn	Used in traditional medicine to treat skin infections, burns and wounds as well as heal broken bones and injured ligaments and tendons in animals	Bharti *et al.* 2014

Eng- English; Shn- Shona

Table 2: Antioxidant activity of extracts from three plant species used as ethno-veterinary medicines in Zimbabwe as determined by the DPPH scavenging assay and *β*-carotene-linoleic acid model system.

Plant species	Plant part	Antioxidant activity		
		DPPH scavenging activity EC_{50} (μg/ml)	ANT (%)	ORR
Erythrinna abyssinica	Leaves	75.57 ± 1.35^{cd}	47.46 ± 1.25^{a}	0.33 ± 0.02^{c}
Erythrinna abyssinica	Bark	**41.30 ± 1.27^{b}**	87.71 ± 1.81^{cd}	**0.09 ± 0.01^{a}**
Cissus quadrangularis	Leaves	**21.04 ± 3.00^{a}**	90.30 ± 2.34^{d}	**0.03 ± 0.01^{a}**
Cissus quadrangularis	Stem	86.69 ± 3.80^{e}	60.10 ± 2.26^{b}	0.32 ± 0.02^{c}
Adenium multiflorum	W/plant	**68.34 ± 1.52^{c}**	63.40 ± 1.53^{b}	**0.16 ± 0.13^{ab}**
Ascorbic acid		71.11 ± 0.01^{d}		
BHT			81.45 ± 1.72^{c}	0.19 ± 0.02^{ab}

Plant extracts with EC_{50} values (< 71.11 μg/mL) and written in bold are considered potent DPPH radical scavengers. The lower the EC_{50}, the more rapidly the colour of DPPH radical was bleached and hence the more potent the antioxidant. ANT (%) - Antioxidant activity calculated on the basis of the rate of *β*-carotene bleaching at $t = 60$ min. ORR - Oxidation Rate Ratio at $t = 60$. The lower the ORR value, the more protective the compound/extract against *β*-carotene bleaching. Cultivar extracts with ORR values ≤ 0.05 and written in bold are considered potent antioxidants. Mean values (± SE) in column with different letters are significantly different ($P < 0.05$; $n = 3$).

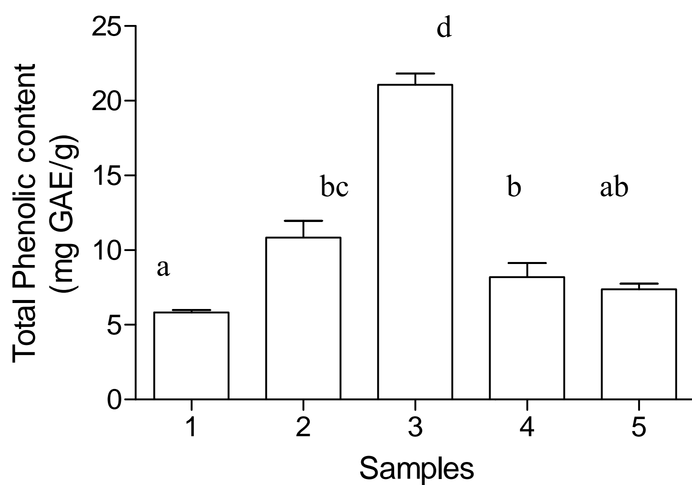

Figure 1: Total phenolic content of extracts from three plant species used as ethno-veterinary medicines in Zimbabwe. Values expressed as gallic acid equivalent (GAE) per gram of plant material. Mean values (± SE) on bar graphs with different letters are significantly different ($P < 0.05$; $n = 4$). 1).*Erythrina abyssinica* Leaves; 2). *Erythrina abyssinica* Bark; 3). *Cissus quadrangularis* Leaves; 4). *Cissus quadrangularis* Stem; 5). *Adenium multiflorum*

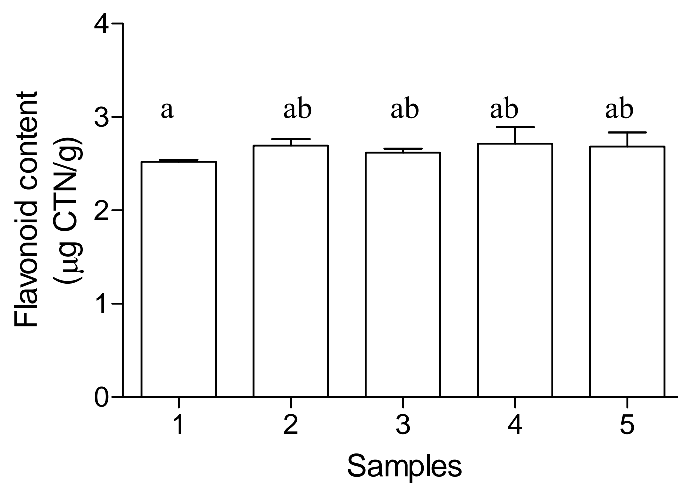

Figure 2. Flavonoid content of extracts from three plant species used as ethno-veterinary medicines in Zimbabwe. Values expressed as catechin equivalents (CAT) per gram of plant extracts. Mean values (± SE) on bar graphs with different letters are significantly different ($P< 0.05$; $n = 4$). 1). *Erythrina abyssinica* Leaves; 2). *Erythrina abyssinica* Bark; 3). *Cissus quadrangularis* Leaves; 4).*Cissus quadrangularis* Stem; 5). *Adenium multiflorum*.

Table 3. Mineral content of three plant species used as ethnoveterinary medicines in Zimbabwe for wound healing based on the ICP-OES method

		Micro nutrients									
Plant species	Plant part	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	B (ppm)
Erythrina abyssinica	Leaves	2.08 ± 0.02	0.13 ± 0.01	0.81 ± 0.01	3.11 ± 0.11	0.70 ± 0.01	360.50 ± 1.50	7.20 ± 0.31	13.25 ± 0.26	127.00 ± 3.00	21.49 ± 1.51
Erythrina abyssinica	Bark	1.55 ± 0.07	0.06 ± 0.01	2.06 ± 0.01	0.55 ± 0.03	0.44 ± 0.05	135.50 ± 2.50	9.37 ± 0.14	199.00 ± 1.00	15.76 ± 0.26	23.50 ± 0.50
Cissus quadrangularis	Leaves	2.74 ± 0.01	0.29 ± 0.01	1.11 ± 0.01	0.49 ± 0.01	0.23 ± 0.01	580.00 ± 10.00	9.12 ± 0.12	36.80 ± 0.30	493.00 ± 8.00	38.12 ± 2.12
Cissus quadrangularis	Stem	1.27 ± 0.06	0.20 ± 0.01	2.18 ± 0.04	4.80 ± 0.11	1.19 ± 0.01	477.50 ± 4.50	10.15 ± 0.15	19.91 ± 0.59	105.50 ± 1.50	27.61 ± 0.61
Adenium multiflorum	W/plant	1.21 ± 0.02	0.68 ± 0.01	3.50 ± 0.04	2.49 ± 0.05	0.68 ± 0.02	1917.00 ± 12.00	8.11 ± 0.11	94.00 ± 1.00	163.00 ± 4.00	33.50 ± 0.50

1 **Table 4: Protein-precipitating activity as a wound healing model of phenolic rich methanolic extracts of three plant species used as**
2 **ethnoveterinary medicines in Zimbabwe for wound healing.**

3

		Protein-precipitating activity		
Plant species	Plant part	Total phenolics (y)*	Protein-precipitating phenolics (x)*	Protein-precipitating capacity (%)
Erythrina abyssinica	Leaves	$0.145 \pm 1.2\times10^{-03}$	$0.122 \pm 1.2\times10^{-03}$	84.138 ± 1.372^{b}
Erythrina abyssinica	Bark	$0.132\pm 1.0\times10^{-02}$	$0.088 \pm 0.6\times10^{-02}$	66.673 ± 0.662^{a}
Cissus quadrangularis	leaves	$0.139\pm 1.0\times10^{-02}$	$0.133\pm 1.0\times10^{-02}$	95.683± 0.123
Cissus quadrangularis	Stem	$0.129\pm 1.0\times10^{-02}$	$0.092\pm 1.0\times10^{-02}$	71.318± 0.211
Adenium multiflorum	W/plant	$0.130\pm 1.0\times10^{-03}$	$0.082\pm 1.0\times10^{-03}$	63.077± 0.033

4

5 *x and y are the slopes of the curve (mg phenolics precipitated/mg plant samples) representing the protein-precipitating and the total phenolics in the sample respectively.

6 Different letters across the protein-precipitating capacity (%) row indicate significant differences($P\leq0.05$) between the protein biding capacities of the extracts from the two sources as separated
7 by the Student *t*-test (n = 3).

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