

Full Length Research

In Vitro Antioxidant Activities and HPTLC Fingerprint Analysis of five Malawian Medicinal Plants.

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Accepted 29th January, 2019.

Antioxidants, as natural compounds have been documented to possess scavenging abilities which helps in fighting body infections. Therefore, the aim of this study was to analyse the phytochemical constituents and antioxidant potential of some medicinal plants reported to possess anti-viral activity in Malawi. The antioxidant potentials of *Aeschynomene nyassana*, *Phyllanthus amarus*, *Euphorbia whyteana*, *Rhus acuminatissima* and *Erica milanjiensis* plants were evaluated using DPPH, FRAP, Reducing power, Nitric oxide scavenging activity and HPTLC while phytochemical analysis was done using spectrophotometric techniques. The phytochemical analysis showed the presence of saponins, flavonoids, alkaloids, phenolic in the plant extracts. The results showed that ascorbic acid had significantly higher DPPH scavenging activity as compared to the plant extracts at all levels. NO scavenging activity demonstrated a significant dose dependent decrease except for *R. acuminatissima*. *A. nyassana* revealed reducing power ability that was significantly greater as compared to Ascorbic acid. In HPTLC analysis, DPPH pale-yellow coloured spots were observed while the phenolic active blue colour spots were observed only in *A. nyassana*, *P. amarus*, *R. acuminatissima* and *E. milanjiensis*. Therefore, this study recommends further research on the 5 plants should be undertaken in order to inform science of traditional use in Malawi.

Keywords: Antioxidant, phytochemical compounds, reactive oxygen species, medicinal plants, High performance thin-layer chromatography, viral infections.

1. INTRODUCTION

The World Health Organisation (WHO) estimates that about 80% of the population living in the developing countries especially in Asia and Africa rely almost exclusively on traditional medicine for their primary healthcare needs due to limited availability of health facilities. In almost all the traditional medical

systems in these countries, the medicinal plants play a major role and constitute their backbone [1].

Viral infections are a major cause of high morbidity and mortality rates in most of these developing countries. The emergence of viral resistance to drugs, as well as the serious adverse effects

induced by antiviral drugs, has caused serious medical problems in many countries, particularly when administered in combination over prolonged treatment periods [2]. Traditionally, medicinal herbs are known to relieve the symptoms of different human diseases, including infectious diseases, and they are acknowledged to have been used for over thousands of years [3].

Many plants contain large amounts of antioxidants which play an important role in absorbing and neutralizing free radicals, quenching singlets and triplets oxygen or decomposing peroxidase. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many infectious diseases. Radical scavenging antioxidants are particularly important in anti-oxidative defense in protecting cells from injury of free radicals especially common in immunosuppressed individuals [4].

Therefore, the aim of this study was to analyse the phytochemical constituents and antioxidant potential of some medicinal plants reported to possess anti-viral activity in Malawi. *Aeschynomene nyassana* is a shrub that can grow up to 120 cm, has reddish-brown viscid stem that arises from woody rootstock and it belongs to the family, *Fabaceae*. Leaves are 2.5 cm long, pinnate in shape with 15-20 pairs of oblong and obtuse leaflets [5]. *Phyllanthus amarus* is an annual herb found in shady places among other common weeds and it belongs to the family, *Euphorbiaceae*. It can grow up to 30-60 cm in height and blooms with yellow flowers. All parts of the plant are used as medicines because of their medicinal properties [6]. *Euphorbia whyteana* is a hairless perennial herb with annual stems that can grow up to 30 cm high from a woody rootstock and it belongs to the family *Euphorbiaceae* which have over 2000 species. Leaves are normally 30 mm long, rounded and apiculate at the apex, they are numerous, sessile, spreading to reflexed and linear-lanceolate. *Rhus acuminatissima* is a small tree often straggling and can grow up to 9 metres in length and it belongs to the family, *Anacardiaceae*. The leaves are 3-foliolate while the leaflets are broadly elliptic, obtuse. The flowers are in loose panicle and small yellowish green in colour. While *Erica milanjiensis* belongs to the family, *Ericaceae* which have over 3000 species consists mainly of shrubs or climbers. It can grow up to 6 metres in length and has greyish brown bark. Leaves are crowded linear, small and dark green in colour [7].

2. MATERIALS AND METHODS

2.1 Chemicals

Trichloroacetic acid (TCA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Diethyl ether, Ammonium hydroxide, Acetic acid, Ethanol, Methanol, Sodium nitroprusside, Sodium bicarbonate, Potassium ferricyanide, Aluminium chloride, Gallic acid, Quercetin, Griess reagent,

Phosphate buffered saline, Folin-Ciocalteu reagent and n-butanol were purchased from Sigma Chemicals Co. (USA). N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA, Ferric chloride and Ascorbic acid were purchased from Merck (Germany). All other chemicals were of analytical grade.

2.2 Preparation of plant materials and extraction

Aeschynomene nyassana, *Phyllanthus amarus*, *Euphorbia whyteana*, *Rhus acuminatissima* and *Erica milanjiensis* medicinal plants were collected and identified by Mr. Edwin Kathumba from Herbarium and Botanical Gardens of Malawi and voucher specimens deposited in the same herbarium. The plants were air-dried at room temperature and then grounded into fine powder. The powdered material was further dissolved twice in methanol for 48 hours. Filtration was conducted using a vacuum system and the filtrates concentrated using Rota vapour (Buchi B-100) to evaporate the solvents from the plant material. The extracts were air-dried at room temperature, weighed and stored for further use.

2.3 Phytochemical analysis

2.3.1 Determination of flavonoid contents

Aluminium chloride method was used to determine flavonoid contents according to standard procedures [8]. About 0.5 ml of 1 mg/ml methanol extract was mixed with 0.5 ml of 2% aluminium chloride, then it was allowed to stand at room temperature for 60 minutes. Absorbance was then measured at 420 nm using the PerkinElmer Victor X3 Multimode plate reader. The total flavonoid content was evaluated as quercetin equivalents (mg/g) using the following equation based on the calibration curve $y = 0.3812x + 0.1257$, $R^2 = 0.9583$, where y was the absorbance and x was the concentration.

2.3.2 Determination of total phenolics

Total phenolic contents were evaluated with Folin-Ciocalteu's phenol reagent according to standard procedures [9]. Five millilitres of the extract solution was mixed with 5 ml Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). The mixture was allowed to stand for 5 minutes, then 4 ml of 7% Na_2CO_3 solution was added. The tubes were vortexed for 15 seconds and allowed to stand for 30 min at 40°C for the development of color. Absorbance was then measured at 765 nm using the PerkinElmer Victor X3 Multimode plate reader. The extracts were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g gallic acid equivalent (GAE) using the following

equation based on the calibration curve: $y = 0.3947x - 0.0423$, $R^2 = 0.981$, where y was the absorbance x was the concentration.

2.3.3 Saponins determination

The saponins content in the plant extracts was determined according to standard procedures [10]. Ten grams of the powdered plant sample was placed in 200 ml of 20% ethanol. The suspension collected was heated for 4 hours in a water bath at 55°C while continuously being stirred. The mixture was filtered and the residue was re-extracted twice as above. The resultant combined extracts were reduced in a water bath at 90°C to about 40 ml. The final concentrate was added to 20 ml diethyl ether in a 250 ml separator funnel and shaken vigorously. The layer of ether solution was discarded, while the purification process was repeated. 60 ml of n-butanol was added and the combined n-butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride. The solution that remained was heated in a water bath to evaporate the solvents and then the sample was dried in the oven to a constant weight. The saponins content was determined according to the equation:

Amount of saponins (mg/g) = weight of residue/weight of sample.

2.3.4 Alkaloids determination

The alkaloids content in the plant extracts was determined according to standard procedures [11]. Five grams of the powdered plant sample was weighed into 200 ml of 20% acetic acid in ethanol and allowed to stand for 5 hours. The extracts were filtered and concentrated using a water bath at 55°C to approximately one-quarter of the original volume. Then dropwise, concentrated ammonium hydroxide solution was added into the resultant extract until precipitation was complete. The precipitate collected after allowing the solution to settle was washed with dilute ammonium hydroxide solution and then filtered. The residue of the crude alkaloid was weighed and calculated according to the equation:

Amount of alkaloid (mg/g) = weight of precipitate/weight of sample.

2.4 Antioxidant assays

2.4.1 Assay of DPPH scavenging activity

The DPPH radical-scavenging activity of the methanol extracts was determined according to standard procedures [12]. DPPH free radical scavenging assay was performed using 96-micro-well flat plates. Stock

solutions of the extracts was prepared as 1 mg/ml in methanol. Each well was filled in with 200 μ l extract of different concentrations (3.13 - 25.0 μ g/ml). Then, 5 μ l of the DPPH solution (2.5 mg/ml in methanol) was added to each well. Ascorbic acid was used as standard control while a blank was prepared by mixing DPPH and methanol. Three replicates were made for each test sample. After 30 minutes of incubation at room temperature in the dark, the optical density of each well was read using PerkinElmer Victor X3 Multimode plate reader at wavelength 517 nm, and results were expressed as percentage antioxidant activity using the following equation:

$100 - [(Sample\ Optical\ density\ (OD) - Sample\ background\ OD) / (DPPH\ only\ OD) \times 100]$.

The IC_{50} values were calculated by plotting a linear regression, where the abscissa represented the concentration of the tested plant extracts and the ordinate represented the average percent of scavenging capacity from three replicates.

2.4.2 Assay of nitric oxide-scavenging activity

The nitric oxide radical scavenging assay was determined according to standard procedures [13]. The extracts were prepared from a 1 mg/mL stock solution of methanol and serially diluted to make concentrations (1.56–50 μ g/mL). Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the methanol extracts (1.56–50 μ g/mL) and incubated at 25°C for 180 mins. The freshly prepared Griess reagent was mixed with equal volume of the plant extracts. Control samples were prepared by mixing equal volume of buffer prepared in a similar manner to the test samples but without the extracts. The colour tubes containing methanol extracts at the same concentrations with no sodium nitroprusside was also prepared. Then, 150 μ L of the reaction mixture was transferred to a 96-well plate and absorbance was measured at 546 nm using a PerkinElmer Victor X3 Multimode plate reader. Ascorbic acid was used as the positive control. The percentage nitric oxide scavenging activity of the methanol extracts and ascorbic acid were calculated using the following formula:

$Nitric\ Oxide\ Scavenging\ activity\ (\%) = A\ control - A\ test / A\ control \times 100.$

2.4.3 Reducing power assay

The reducing power of the plant extracts was determined according to standard procedures [14].

Different amounts of plant extracts (3.125 - 50 µg/ml) in methanol were prepared and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v) and the mixture centrifuged at 3000 rpm for 10 minutes. About 2.5 ml of the supernatant was mixed with an equal volume of distilled water and 0.5 ml of FeCl₃ (0.1% w/v) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive controls.

2.4.4 Ferric-Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was evaluated according to standard procedures [15]. The FRAP reagent was prepared by mixing acetate buffer (25 mL, 300 mmol/L, pH 3.6), TPTZ solution (2.5 mL, 10 mmol/L) in 40 mmol/L HCl and FeCl₃ solution (2.5 mL, 20 mmol/L) in proportions of 10:1:1 (v/v), respectively. The freshly prepared FRAP reagent was warmed at 37°C in a water bath prior to use. Then 150 µl of the sample was added to the 4.5 mL of FRAP reagent. The absorbance of the reaction mixture was measured at 595nm. The standard curve for analysis was constructed using FeSO₄ solution (0.5-10 mg/mL). The results obtained were expressed as µmol Fe (II)/g dry weight of plant material. Ascorbic acid was used as a control.

2.4.5 High Performance Thin Layer Chromatography (HPTLC) study of Phenol and DPPH

High performance thin-layer chromatography (HPTLC) was performed on a silica gel glass plate (20 × 20 cm, Silica gel 60 F254, Merck) according to standard procedures [16]. The extracts were dissolved in ethyl acetate and were directly deposited on glass silica gel. TLC plates were developed in a sandwich TLC chamber with Ethyl acetate/Methanol/Water (10:1.35:1) solvent mixture as mobile solvent. The profiles of the separated spots were sprayed with 90:10 methanol/sulphuric acid reagent after visualization under UV (366 nm) and visible light respectively. The plates were further examined for DPPH active spots. After 25 min, the pale-yellow spots on purple background indicated spots antioxidant activity. A separate run of the plates were stained with Folin-Ciocalteu's reagent and heated at 80°C/10min. The plates were further examined for phenolic active spots. After 25 min, the blue colour spots indicated presence of phenolic compounds.

2.5 Statistical Analysis

All experiments analysis were done in triplicates and where applicable, the data were subjected to one

way analysis of variance (ANOVA). P Values < 0.05 were regarded as significant.

3 RESULTS AND DISCUSSIONS

As shown in Figure 1, significant amounts of saponins (P < 0.05) were quantitatively indicated from *P. amarus* (514.24 mg/g) and *R. acuminatissima* (509.90 mg/g) as compared to 209.9, 370.82 and 167.60 mg/g indicated from *A. nyassana*, *E. whyteana* and *E. milanjeinsis* respectively. Saponins are known to have characteristics that includes bitterness, formation of foams in aqueous solutions, hemolytic activity, antifungal and cholesterol binding properties [19]. Significant amounts of flavonoids (P < 0.05) were also indicated from *E. whyteana* (579.46 mg/g) as compared to 127.51 mg/g indicated from *A. nyassana*. Literature indicates that flavonoids decrease free radicals by chelating radical intermediate compounds and through quenching, up-regulating or protecting antioxidant defences [17]. There were more alkaloids compounds (P < 0.05) indicated from *E. whyteana* (766.90 mg/g) and *E. milanjeinsis* (698.48 mg/g) respectively as compared to the other plants. Alkaloids have been shown to exhibit marked physiological activity when administered to animals and also they are reported to have analgesic activities [18]. Significant amounts of phenolic compounds (P < 0.05) were quantitatively indicated from all the 5 plants under study. Literature indicates that phenolic compounds can readily donate hydrogen atom to the radical and this could be responsible for the DPPH radical scavenging activity reported in the study [13].

In Figure 2A, the results of DPPH scavenging activity demonstrated an inhibition percent of above 80 (87.7, 99.6, 95.7, 81.1, and 94.3) at a higher concentration of 500 µg/ml, and also showed considerably higher inhibition percent of above 50 (70.5, 65.8, 58.3, 62.6 and 67.0) at lower concentration of 7.8 µg/ml for *A. nyassana*, *E. whyteana*, *E. milanjeinsis*, *R. acuminatissima* and *P. amarus* respectively. However, it can be observed that Ascorbic acid had stronger activity as compared to the plants under study and this might be attributed to the additive or synergistic effects of polyphenols making the antioxidant activity of the extracts weaker than that of the isolated bioactive compounds [21].

In Figure 2B, the results of FRAP scavenging activity showed increased absorbance with increased concentration trend for all the plants. The absorbance increased from 0.1 to 0.8, 0.1 to 0.3, 0.2 to 0.5, 0.03 to 0.4, 0.07 to 0.8 and 0.2 to 1.9 for *A. nyassana*, *E. whyteana*, *E. milanjeinsis*, *R. acuminatissima*, *P. amarus* and Ascorbic acid respectively at a concentration range of 0.16-0.5 µg/ml. Literature indicates that compounds with iron chelating ability can act as powerful antioxidants in most of the times. The iron ions are

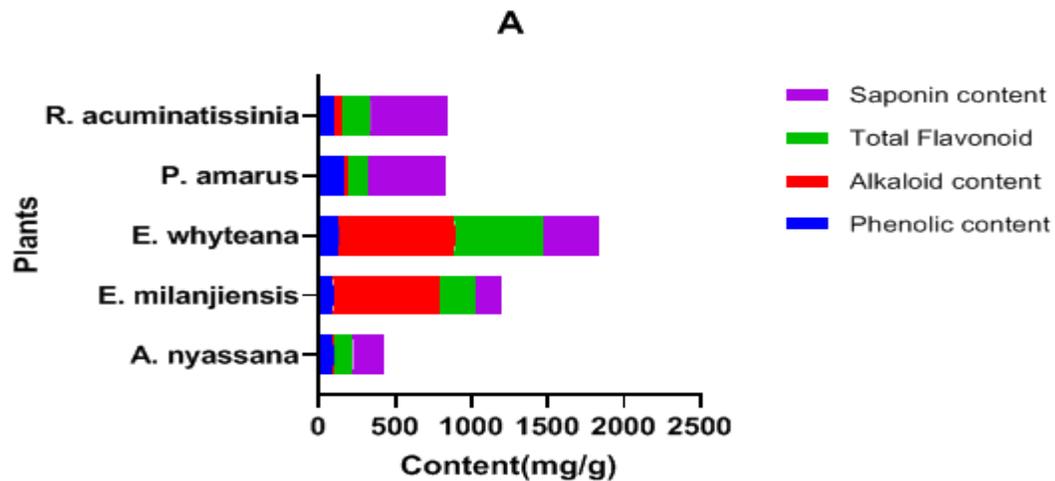


Figure.1: Phytochemical contents for the five medicinal plants were evaluated. Aluminium chloride method was used to assess the flavonoids contents, Folin-Ciocalteu reagents method was used to evaluate the total phenolic contents, Harborne method was used to measure the alkaloids content and also saponins contents were also evaluated. The samples were analysed in triplicate presented as mean \pm standard deviation (SD)

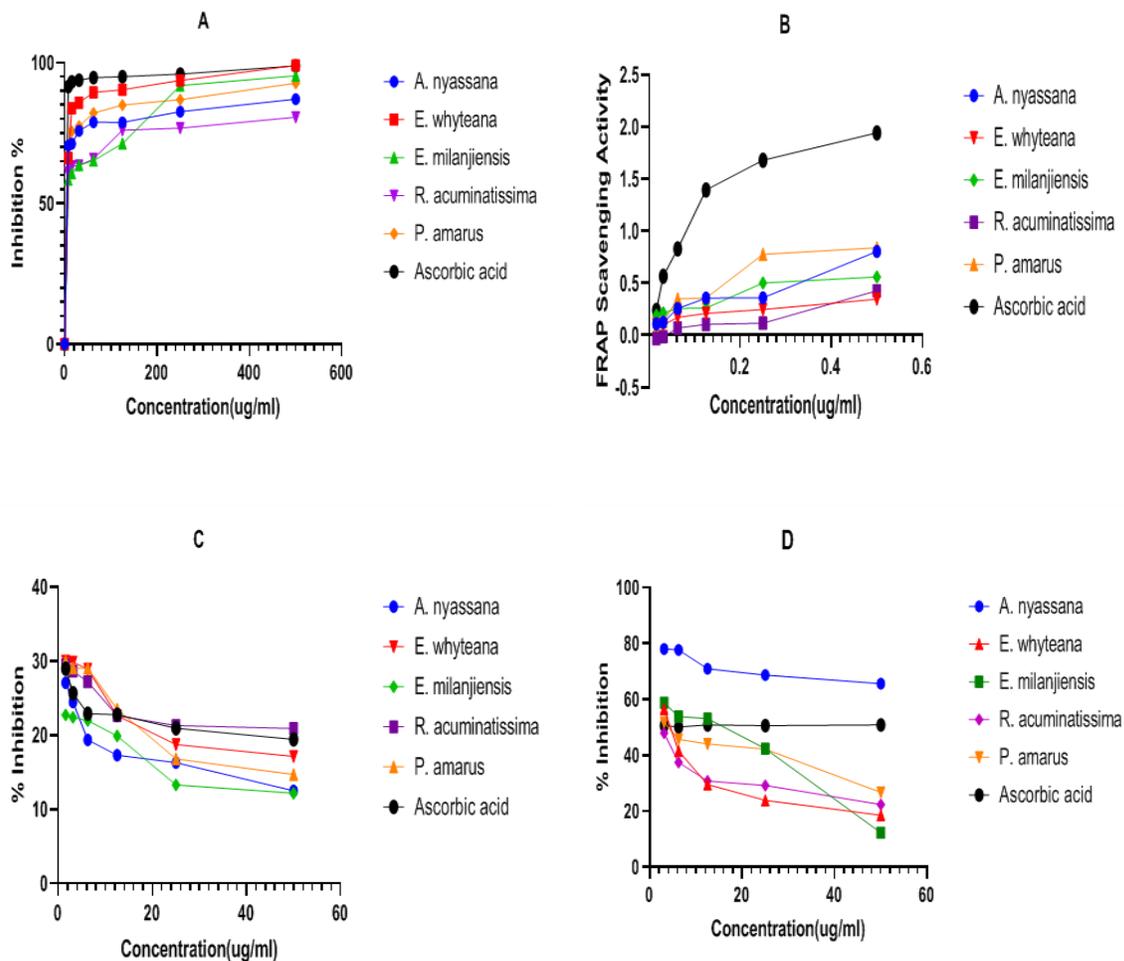


Figure 2: The analysis of 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging, the ferric ion reducing capacity (FRAP), Nitric oxide scavenging and Reducing power activities are shown in Figure 2 A, B, C, and D respectively. The results showed that the five plants extracts had moderate scavenging activity as compared to ascorbic acid at all concentration in all the experiments. Results are means of 3 replicates

known to catalyse the conversion of less reactive species such as lipid peroxides to more reactive species such as hydroxyl, peroxy/alkoxy radicals. And release of iron by cellular damage can accelerate oxidative damage to tissues[23].

In Figure 2C, the nitric oxide scavenging activity for the plant extracts and ascorbic acid demonstrated the scavenging activity of less than 50% inhibition for all the plants under study. *P. amarus*, *R. acuminatissima*, *E. whyteana*, and Ascorbic acid showed lower percentage inhibition of 29.8, 29.4, 29.2 and 28.9 respectively at a concentration of 50 µg/ml. A dose-dependent decrease in NO scavenging activity was observed for all the extracts except *R. acuminatissima* at all the tested concentrations. In the human body, nitric oxide is generated from the amino acid, L-arginine, by vascular endothelial cells, phagocytes and certain cells of the brain. Nitric acid is classified as free radical, because of its unpaired electron and normally displays important reactivity with certain types of proteins and other free radicals. It becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxy nitrite anion hence being toxic [20].

In Figure 2D, the results for reducing power activity displayed an inverse relationship between concentration and percentage inhibition of the plant extracts. *A. nyassana*, *E. milanjiensis*, *E. whyteana*, and ascorbic acid showed reducing power of 78.0, 58.8, 56.5 and 50.8% respectively at 50 µg/ml concentration. Increased absorbance of the reaction mixture indicates an increased reducing power of the plant extracts. The reducing properties of plant extracts has been shown in literature to exert antioxidant action that breaks the free radical chain through donation of a hydrogen atom to the compound [22],

In Table 1, the results showed that the IC₅₀ values for DPPH free radical scavenging activity were within the range of 0.64–3.11 µg/ml, with Ascorbic acid, *E. milanjiensis*, and *E. whyteana* showing a significantly marked IC₅₀ values of 0.64, 1.89 and 1.99 µg/mL respectively. The IC₅₀ values of the DPPH tested samples were in the order: Ascorbic acid < *E. milanjiensis* < *E. whyteana* < *P. amarus* < *A. nyassana* < *R. acuminatissima*

Table 1: The IC₅₀ values for DPPH, Nitric Oxide and Reducing Power of plant extracts

Sample	DPPH		Nitric Oxide		Reducing Power	
	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²
<i>A. nyassana</i>	2.32	0.52	7.47	0.97	9.56	0.94
<i>E. whyteana</i>	1.99	0.69	5.45	0.87	2.43	0.77
<i>P. amarus</i>	2.26	0.51	4.27	0.89	0.43	0.29
<i>R. acuminatissima</i>	3.11	0.59	8.90	0.92	0.17	0.94
<i>E. milanjiensis</i>	1.89	0.75	9.74	0.85	2.42	0.77
Ascorbic acid	0.64	0.39	11.47	0.94	1.82	0.52

IC₅₀ values are half maximal inhibitory concentration which is a measure of the potency of a substance in inhibiting a specific biological or biochemical function while R² is a statistical measure of how close the data are to the fitted regression line. The IC₅₀ values and R² were calculated for DPPH, Nitric oxide and Reducing power assays for all the plants under study

For nitric oxide scavenging activity, the IC₅₀ values were within the range of 5.45–11.47 µg/mL with *R. acuminatissima* and *E. milanjiensis* significantly showing the IC₅₀ values of 8.90 and 9.74 µg/mL respectively as compared to 11.47 for Ascorbic acid. The IC₅₀ values of the nitric oxide scavenging activity tested samples were in the order: *P. amarus* < *E. whyteana* < *A. nyassana* < *R. acuminatissima* < *E. milanjiensis* < Ascorbic acid

For the reducing power assay, the IC₅₀ values were within the range of 0.43–9.56 µg/mL with *A. nyassana*, *E. whyteana*, and *E. milanjiensis* showing IC₅₀ values of 9.56, 2.43 and 2.4 respectively above the reference ascorbic acid. The IC₅₀ values of the reducing power assay tested samples were in the order: *R. acuminatissima* < *P. amarus* < Ascorbic acid < *E. milanjiensis* < *E. whyteana* < *A. nyassana*.

As shown in Table 2, the presence of antioxidant substances were demonstrated by a change in colour to pale-yellow colour spots on purple background and these changes were observed in *A. nyassana*, *E. whyteana*, *P. amarus*, *R. acuminatissima* and *E. milanjiensis* plants. While in a separate HPTLC run of plates stained with Folin-Ciocalteu reagent, the presence of phenolic compounds were demonstrated by change to blue colour of spots with yellow background. The changes were observed in *E. milanjiensis* (0.69, 0.75, 0.88, 0.94, 0.97), *E. whyteana* (0.75, 0.88, 0.94, 0.97), *P. amarus* (0.56, 0.67, 0.97), *R. acuminatissima* (0.38, 0.94, 0.97) and *A. nyassana* (0.97).

Table 2: High-Performance Thin-Layer Chromatography(HPTLC) separation of the extracts

Plant	Rf value	Number of spots	Colour Observation methanol/sulphuric acid	Folin-Ciocalteu
<i>A. nyassana</i>	0.97	1	Pale-yellow	Blue colour
<i>E. whyteana</i>	0.75, 0.88, 0.94, 0.97	4	Pale-yellow	-
<i>P. amarus</i>	0.56, 0.67, 0.97	3	Pale-yellow	Blue colour
<i>R. acuminatissima</i>	0.38, 0.94, 0.97	3	Yellow	Blue colour
<i>E. milanjiensis</i>	0.69, 0.75, 0.88, 0.94, 0.97	5	Pale-yellow	Blue colour

Chromatographic separation of the extracts was developed using ethyl acetate/methanol/water (10:1.35:1), the profiles of separated spots sprayed with 90:10 methanol/sulphuric acid reagent after visualization under UV (366 nm) and visible light respectively. For phenolic compounds, a separate HPTLC run was done in which plates were stained with Folin-Ciocalteu reagent to assess the phenolic compounds.

4 CONCLUSIONS

The presence of phytochemical compounds such as phenolic, alkaloids, flavonoids, saponins and the inhibitory effect of *A. nyassana*, *E. milanjiensis*, *E. whyteana*, *P. amarus* and *R. acuminatissima* extracts on the free radicals provides some scientific evidence on the traditional usage in the management of opportunistic infections. These phytochemical compounds provides scavenging abilities which assist the body to fight infections. However, further studies on these plants are recommended to be undertaken in order to inform science.

Conflicts of interest

The authors have no conflicting of any interest.

ACKNOWLEDGEMENT

Grateful thanks goes to the Department of Biomedical Sciences at College of Medicine, University of Malawi for providing facilities for research work. The authors also thank the ACEPHEM project for providing invaluable support during the research work.

REFERENCES

1. WHO Traditional medicine. 2008 World Health Organization. Available online: <http://www.who.int/mediacentre/factsheets/2003/fs134/en/>
2. Yin PD, Das D, Mitsuya H(2006). Overcoming HIV drug resistance through rational drug design based on molecular, biochemical, and structural profiles of HIV resistance. *Cell Mol. Life Sci.* 63: 1706-1724.
3. Kitazato K, Wang Y, Kobayashi, N(2007). Viral infectious disease and natural products with antiviral activity. *Drug Discov. Ther.* 1: 14-22.
4. Lu J, Lin PH, Yao Q(2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J. Cell Mol. Med.* 14: 840-860
5. Hyde, M.A.; Wursten, B.T.; Ballings, P.; Coates Palgrave, M. *Flora of Malawi: Species information: Aeschynomene nyassana*. 2018. https://www.malawiflora.com/speciesdata/species.php?species_id=130570, retrieved 11 July 2018.
6. Shetti AA, Sanakal RD, Kaliwal BB (2012). Antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice. *Asian J. Plant Sci. Res.* 2:11-15.
7. Morris B, Msonthi JD(1996). *Chewa Medical Botany: A Study of Herbalism in Southern Malawi*. Vol 2 of Monographs from the International African Institute, International African Institute.
8. Ordonez AAL, Gomez JD, Vattuone MA, Isla MI (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry* 97: 452-458.
9. Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* 51: 609-614. 10.1021/jf020782a.
10. Kim DO, Jeong SW, Lee CY(2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81: 321-326. 10.1016/S0308-8146(02)00423-5.
11. Obadoni BO, Ochuko PO(2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta State of Nigeria. *Global Journal of Pure and Applied Sciences* 8: 203-208.
12. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M(2005). Antimicrobial and antioxidant activities of the essential oil and various extracts

- of *Salvia tomentosa* Miller (Lamiaceae). Food Chemistry 90: 333–340.
13. Tung YT, Wub HJ, Hsieh C, Ping-Sheng, Chen PS, Chang ST (2009). Free radical-scavenging phytochemicals of hot water extracts of *Acacia confusa* leaves detected by an on-line screening method. Food Chemistry 115: 1019-1024.
 14. Yen GC, Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry 43: 27-32.
 15. Jimenez-Alvarez D, Giuffrida F, Vanrobayes F, Golay PA, Otting CC, Ardeau AL, et al. (2008). High-throughput methods to assess lipophilic and hydrophilic antioxidant capacity of food extracts in vitro. Journal of Agricultural and Food Chemistry 56: 3470–3477.
 16. Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM (2009). In Vitro Antioxidant and Xanthine Oxidase Inhibitory Activities of Methanolic *Swietenia mahagoni* Seed Extracts. Molecules 14: 4476-4485.
 17. Ndhlala AR, Finnie JF, Van Staden J (2010). In vitro antioxidant properties, HIV-1 reverse transcriptase and acetylcholinesterase inhibitory effects of traditional herbal preparations sold in South Africa. Molecules 15: 6888-6904.
 18. Okwu DE, Okwu ME (2004). Chemical composition of *Spondias mombin* linn plant parts. J. Sustain Agric. Environ. 6: 140-147.
 19. Sodipo OA, Akiniyi JA, Ogunbamosu JU (2000). Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K schemp) pierre Exbeille. Glob. J. Pure Appl. Sci. 6: 83-87.
 20. Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR (2011). Antioxidant activity and free radical scavenging potential of *Pithecellobium dulce* Benth seed extracts. Free Radicals and Antioxidants 2: 37–43. doi: 10.5530/ax.2012.2.2.7,
 21. Hayouni EA, Abedrabba M, Bouix M, Hamdi M (2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 105: 1126-1134. 10.1016/j.foodchem.2007.02.010.
 22. Erukainure OL, Oke OV, Ajiboye AJ, Okafor OY (2011). Nutritional qualities and phytochemical constituents of *Clerodendrum volubile*, a tropical non-conventional vegetable. Intern. Food Res. J. 4: 1393-1399.
 23. Halliwell B (2009). The wanderings of a free radical. Free Radic. Biol. Med. 46:531–542.