CONSORTIUM

History:
Received: June 22, 2014
Accepted: July 12, 2014
First Published: October 1, 2014
Collection year: 2014
Confirmation of publication: Published

Identifiers and Pagination:
Year: 2014
Volume / Issue: 6/4
First Page: 332
Last Page: 343
Publisher Id: JAppPharm-6-4
DOI:http://dx.doi.org/10.21065/19204159

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Citation:
Foo Rui Qing, Manogaran Elumalai, Gabriel Akyirem Akowuah. Antimicrobial and antioxidant studies of vernonia amygdalina. J App Pharm (2014) 6:4 332-343.

Original Research Article

ANTIMICROBIAL AND ANTIOXIDANT STUDIES OF *VERNONIA AMYGDALINA*

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ABSTRACT

In this study, the ethanol extract (EE), ethyl acetate extract (EAE), aqueous decoction extract (ADE) and aqueous maceration extract (AME) of *Vernonia amygdalina* leaves were subjected to total phenolic (TP) and total saponin (TS) content determination, *in vitro* radical scavenging activity and antimicrobial susceptibility testing (AST). The TP contents in order of decreasing quantities were 63.044, 38.834, 53.148 and 39.391 mg of gallic acid equivalents (GAE) per g dry extract for EE, EAE, ADE and AME, respectively. The TS assay revealed that the EAE extract possessed the highest TS content with a value of 952.037 mg of diosgenin equivalents (DE) per g dry extract. This was followed by the EE, ADE and AME extract with TS content of 841.370, 159.741 and 118.444 mg diosgenin equivalents (DE) per gram dry extract. The DPPH assay revealed that the ADE had the highest DPPH radical scavenging activity with an IC_{50} value of 501.207 µg/ml. This was followed by EE, EAE and AME with IC_{50} values of 636.010, 658.277 and 1368.929 µg/ml, respectively. The ADE also exhibited the highest ABTS radical scavenging activity with an IC_{50} value of 3195.083 µg/ml. In the subsequent order of decreasing ABTS scavenging activity was AME, EE and EAE extracts with IC_{50} values of 4142.156, 5508.517 and 6547.940 µg/ml. The results of AST showed that the test organisms *E. coli* O157:H7 and *Y. enterocolitica* were resistant towards the *V. amygdalina* extracts tested. Only the AME extract possesses a MIC value of 31.25 mg/ml against *Y. enterocolitica*. The data obtained reaffirms *V. amygdalina' s*pontential as a source of dietary antioxidants and provides information on the the suitability of *V. amygdalina* against *E. c.oli* O157:H7 and *Y. enterocolitica.*

Key words:*V. amygdalina*; total phenolic; total saponin; free radical; antimicrobial

INTRODUCTION

There are many reports attesting to the antimicrobial properties of plants due to the presence of secondary metabolites.[1] With the numbers of drug resistant pathogens constantly on the rise, more and more researchers are focusing their research on natural products with the hope of discovering new therapeutic compounds.[2] Similarly, plants are known to possess antioxidant properties with most of them attributed to the phenolic compounds found in plants.[3]The presence of antioxidants in plants is important as many plants are used as a source of dietary antioxidants.[4]

Vernonia amygdalina is a plant that is commonly cultivated in Africa for food as well as for its medicinal properties[5]; and there have been several reports on the plant's antioxidant and antimicrobial properties.[6,7] Previous studies[8,9] have reported DPPH IC_{50} values for aqueous and ethanolic extracts of *V. amygdalina* but there have been no reports on the IC_{50} values for the ABTS free radical scavenging assay using *V. amygdalina* extracts. Owhe-Uregheet *al.*[10] reported antimicrobial properties of aqueous and ethanolic extracts of *V. amygdalina* against *Y. enterocolitica*. At present, there are no reports on the antimicrobial activity of *V. amygdalina* against *E. coli* O157:H7. The present study describes antimicrobial properties of ADE, AME, EE and EAE leaf extracts of *V. amygdalina* against two food borne pathogens; *E. coli* O157:H7 and *Y. enterocolitica*. The study also includes the antioxidant properties of the leaf extracts which were reassessed through IC_{50} studies using the DPPH and ABTS free radical scavenging assay.

CONSORTIUM

Reviewing editor:
Taha Nazir, Ph.D. Scientific Executive ICDTD Inc. Saskatoon Saskatchewan Canada.: E. taha@icdtdi.ca

Funding:
The authors received no direct funding for this research.

Competing Interests:
The authors declare no competing interests

Additional information is available at the end of the article.

Table 1 Amount (g) and % yield of extracts of *Hypericum ericoides*

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, Sodium carbonate, methanol and ethyl acetate were from Merck (Germany). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Sigma-Aldrich (Canada). Gallic acid, 2,2-diphenyl-1-picryhydrazyl (DPPH) and Butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich (USA). Ethanol and dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (UK). Vanillin and trolox were purchased from Acros Organics (USA). Sulphuric acid was purchased from Friendemann Schmidt Chemical (Australia). Diosgenin was purchased from ChromaDex (USA).

For antimicrobial studies, Mueller-Hinton agar (MHA), ampicillin (10 µg/disc) and chloramphenicol (30 µg/disc) were purchased from Oxoid (UK). Chloramphenicol powder was purchased from Bio Basic (Canada) and Mueller-Hinton Broth (MHB) was purchased from Difco (USA).

Test microorganisms

Escherichia coli O157:H7 vegetable and chicken isolates were obtained from the Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM). *Yersinia enterocolitica* ATCC 23715 was purchased from Thermo Scientific, USA. The test microorganisms were maintained on TSA agar plates and stored at 2-8 ºC.

Plant materials

The *V. amygdalina* leaves (8.564 kg) were collected from the Yik Poh Ling Tropical Herbal Farm, Negeri Sembilan on the 17th of September 2012 and a sample of the leaves were sent to UPM for identification. Back in the laboratory, the leaves were weighed and then rinsed to remove the surface dirt and contaminants before being air dried in the shade for a week. After drying, the leaves were weighed once again and the moisture content was determined. The dried leaves were then ground into powder using a commercial blender (Waring Products Division, USA). The powder was stored in sealed plastic containers until needed.

Plant extraction

The ADE extract of *V. amygdalina* were prepared according to the method described by Ola *et al*.[11] with slight modifications. The powdered *V. amygdalina* leaves (10 g) were boiled in 150 ml ultrapure water (ElgaLabwater, UK) for 10 min before being allowed to cool. After cooling, the solution was filtered through a double rings filter paper using a Buchner funnel and rinsed to bring the volume up to 150 ml. After rinsing, the extract was freeze dried (Martin Christ, Germany) and stored in a desiccator for future use.

The AME, EE and EAE extracts were prepared according to the method used by Margeretha *et al*.[12]. Briefly, 100 g of powdered *V. amygdalina* leaves was placed into three separate 1000 ml Erlenmeyer flasks. Then, 900 ml of ethanol was added to the first flask, 900 ml of ethyl acetate was added to the second flask and 600 ml of ultrapure water was added to the third flask. These flasks were then wrapped in aluminum foil and periodically shaken at room temperature. After 24 h, the ethanol and ethyl acetate solutions from these flasks were filtered using a double rings filter paper and the filtrate concentrated using a rotary vacuum evaporator (Tokyo Rikakikai, Japan)at 45-50 ºC. To the marc, a fresh volume of solvent was added and the maceration continues for another 24 h. The maceration process, followed by concentration via rotary vacuum evaporator, was repeated for 14 days until the addition of the solvent to the marc produces a clear solution. The obtained concentrated filtrates were then combined before the final drying in an oven at 40 ºC to produce a constant weight. The extraction yields of the crude EE and EAE extracts were determined and the extracts were stored in a desiccator at room temperature.

The AME extract, after filtering through a double rings filter paper, was freeze dried. Fresh ultrapure water was then added to the marc and the maceration-freeze drying process was repeated for ten

days until a clear solution was obtained. The freeze dried crude extracts were then combined and the extraction yield was determined. The AME extract was then stored in a desiccator at room temperature.

Total phenolic content determination

The TPC assay was done according to the method described by Atanassova *et al.*[13] with minor modifications. The assay was carried out by mixing 0.2 ml of sample solution (consisting of either plant extract or gallic acid standard) with 1.8 ml of ultrapure water and 0.2 ml of Folin-Ciocalteu reagent (10X dilution). The mixture was then allowed to incubate for 5 min before 2 ml of 7% aqueous Na_2CO_3 solution and 0.8 ml of ultrapure water was added. After 90 min of incubation, the absorbance was measured at 750 nm using a Lambda 25 UV/Vis Spectrometer (Perkin Elmer; US). For the reagent blank, the plant extract/ gallic acid standard was replaced with 80% aqueous methanol. To prepare the calibration curve, gallic acid was assayed at concentrations 10, 20, 40, 60, 80 and 100 µg/ml. The TPC values of the plant extracts (1 mg/ml) were determined by substituting the absorbance values obtained into the linear equation generated from the standard curve. The TPC assay for each extract was performed in triplicates and expressed as milligrams of gallic acid equivalents (GAE) per g of dry extract.

Total saponin content determination

The total saponin assay was performed according to the method given by Makkar*et al*.[14] The assay was performed by mixing 0.25 ml of sample solution (consisting of either plant extract or diosgenin standard) with 0.25 ml of 8% vanillin reagent and 2.5 ml of 72% (v/v) H_2SO_4. The solution was mixed thoroughly before being placed into a hot water bath at 60 ºC for 10 min. After heating, the solution was cooled in ice-cold water for 3-4 min before the absorbance was read at 544 nm. For the reagent blank, the sample solution was replaced with 80% aqueous methanol. The calibration curve was prepared by assaying the diosgenin standard at 50, 100, 150, 200, 300, 400 and 500 µg/ml. The total saponins content of the extracts (1 mg/ml) were quantified by substituting the absorbance values of the plant extracts into the linear equation generated by the standard curve. The total saponin assay was done in triplicates for each plant extract and the values were expressed as milligrams of diosgenin equivalents (DE) per g of dry extract.

***In vitro* free radical scavenging assay**

a) DPPH radical scavenging assay

The DPPH radical scavenging activity was done according to the method used by Sharma and Goyal.[15] In each test tubes 1 ml of sample solution (containing antioxidant from either the plant extract or BHA standard) and 6 ml of methanolic DPPH solution (100 µM) were mixed together and incubated in the dark for 10 min. After incubation the decrease in absorbance was measured at 517 nm using a Lambda 25 UV/Vis Spectrometer and the % DPPH radical scavenging activity was calculated using Equation 1. Methanol was used as the reagent blank.

Equation 1:

DPPH radical scavenging capacity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample solution.The IC_{50} values of each sample were determined from the polynomial equation generated from the plotted curve using the Microsoft Office Excel 2007 program. The IC_{50} is defined as the concentration of the sample that is required to scavenge 50 % of the DPPH radical. DPPH assay was carried out in triplicates. BHA was used as positive control.

b) ABTS radical scavenging assay

The $ABTS^{\cdot+}$radical scavenging assay was done according to the method employed by Re *et al*. and

Teow*et al*.[16, 17] with slight modifications. The $ABTS^{\cdot+}$ solution was prepared by mixing 192.2 mg of ABTS salt with 33.2 mg of potassium persulfate in 50 ml of ultrapure water and this will give the solution a final concentration of 7 mM ABTS and 2.456 mM potassium persulfate. The $ABTS^{\cdot+}$ solution was then stored in the dark at room temperature for 16 h before use. The $ABTS^{\cdot+}$ radical scavenging assay was performed by first diluting the $ABTS^{\cdot+}$ solution at room temperature with 95% ethanol for the Trolox standard, EE and EAE extract; and 76% ethanol for the ADE and AME extracts to an absorbance of 0.70 ($\pm$ 0.05) at 734 nm (approximately 7 ml $ABTS^{\cdot+}$ solution in 500 ml 95% ethanol). After dilution, 4 ml of diluted $ABTS^{\cdot+}$ solution is placed into each test tube and 40 µl of sample solution (containing either Trolox standard or plant extract) is added. The solution is mixed and the decrease in absorbance was measure at 734 nm after 6 min. For the reagent blank, 95% ethanol was used for the Trolox standard, EE and EAE extract while 76% ethanol was used for the ADE and AME extracts. The ABTS radical scavenging activity was determined using Equation 2.

Equation 2:

$$\text{ABTS radical scavenging capacity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample solution. The IC_{50} values of each sample were determined from the polynomial equation generated from the plotted curve using the Microsoft Office Excel 2007 program. The concentration at which 50% of the ABTS radical is scavenged is known as the IC_{50}. The ABTS IC_{50} assay was carried out in triplicates.

Antimicrobial susceptibility test

a) Antimicrobial screening

The EE extract (500 mg/ml) and EAE extract (500 mg/ml) were prepared by dissolving 500 mg of crude extract into 1 ml of 99.99% DMSO. The ADE extract (500 mg/ml) and AME extract (500 mg/ml) were prepared by dissolving 500 mg of crude extract into 1 ml of sterilised ultrapure water. The ADE and AME extracts were then filtered through a 0.45 µm nylon filter under a laminar flow hood for sterilisation before use.

The screening of the antimicrobial properties of *V. amygdalina* was done using the disk diffusion method according to the protocol given by the Clinical and Laboratory Standards Institute (CLSI).[18] The preparation of the inoculum suspension was done using 24-hour-old cultures and adjusted to that of a 0.5 McFarland standard. The cell suspension was then streaked onto Mueller-Hinton agar (MHA) plates. After streaking, 6 mm antibiotic assay discs impregnated with 25 µl of plant extracts (500 mg/ml) were placed onto the agar surface. The MHA was then inverted and incubated at 35 $\pm$ 2 ºC under ambient air for 16-18 h. After incubation, the inhibition zones were measured to the nearest millimetre with a ruler and recorded. Extracts exhibiting antimicrobial activity were then chosen for the minimum inhibitory concentration (MIC) assay. For negative controls, antibiotic assay discs containing 99.99% DMSO and sterilised ultrapure water was used. For positive controls, ampicillin (10 µg/disc) was used for the *E. coli* O157:H7 strains while chloramphenicol (30 µg/disc) was used for *Y. enterocolitica* ATCC 23715. Aseptic conditions were maintained throughout the antimicrobial susceptibility screening and all relevant apparatus were autoclaved prior to use. The antimicrobial screening was done in triplicates.

b) Determination of minimum inhibitory concentration (MIC)

The MIC determination was done using the broth macrodilution method.[19, 20] Chloramphenicol stock solution (positive control) was subjected to two fold dilutions to give concentrations of 128, 64, 32 and 16 µg/ml. From these prepared concentrations, 0.5 ml of the antibiotic was transferred into screw capped test tubes and after the addition 0.5 ml of inoculum into these test tubes (1:2 dilution), the final concentration of the chloramphenicol solutions were 64, 32, 16 and 8 µg/ml, respectively. Likewise, the AME extract was subjected to two fold dilutions to give concentrations of 500, 250, 125, 62.5 and 31.25 mg/ml. Then, 0.5 ml of the plant extract was transferred into screw capped test tubes and after the addition 0.5 ml of inoculum into these test tubes (1:2 dilution), the final concentration of

the AME extract were 250, 125, 62.5, 31.25 and 15.625 mg/ml, respectively.

The MIC assay was done using 24-hour-old *Y. enterocolitica* cultures. The inoculum was prepared by adjusting the *Y. enterocolitica* suspension in 0.85% NaCl to match that of a 0.5 McFarland standard turbidity. This gave a cell density of about 1 x 10^8 CFU/ml. Then, the *Y. enterocolitica* suspension was further diluted (1:100) with CAMBH to give a cell density of 1 x 10^6 CFU/ml. Within 15 min of preparation, 0.5 ml of the inoculum was added into the test tubes containing the antimicrobial solution. This gave the inoculum a final concentration of 5 x 10^5 CFU/ml.

For controls, the growth control consisted of 0.5 ml of test inoculum with 0.5 ml of sterile water. For the broth control tube, 0.5 ml of CAMBH with 0.5 ml of sterile water was mixed together. An additional tube containing 0.5 ml of AME extract and 0.5 ml of CAMBH was also included with the assay to test for the sterility of the extract. To check for the purity of the test organism, 10 µl of suspension was removed from the growth control tube and streaked onto a TSA plate.

Incubation of the samples, controls and purity tests were done at 35 ± 2 ºC under ambient air for 16-20 h. After incubation, the MIC was determined by visually checking the test tubes after gentle agitation. The lowest concentration of antimicrobial agent/extract that completely inhibits the growth of the test organism (detected by lack of visual turbidity) is known as MIC and the values were reported in mg/ml. For the plant extract, the MIC assay was done in triplicates. Aseptic conditions were maintained throughout the MIC assay and all relevant apparatus were autoclaved prior to use.

Statistical analysis

The statistical analysis was done using the one-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test on the Prism Graph Pad software. The difference between the means at 5% confidence level was considered significant. The linear regression analysis was done using Microsoft Excel 2007 to compare the correlation between the total phenolics and total saponin against the free radical scavenging activities of the *V. amygdalina* extracts.

RESULTS AND DISCUSSION

Moisture content and extraction Yield

The moisture content of *V. amygdalina* leaves was 83.080%. Figure 1 shows the extraction yields of *Vernonia amygdalina* leaf using different solvent. The ADE extract had the highest extraction yield with a value. The extraction yield of the samples exhibited the descending order: ADE > AME > EE > EAE. The different extraction yields obtained indicates that the use of different extraction solvents affects the extraction yield. Depending on the type of extraction solvent used, different bioactive compounds may be recovered based on their chemical characteristics and polarities with polar solvents used for extracting polar compounds and vice versa.[21,22]

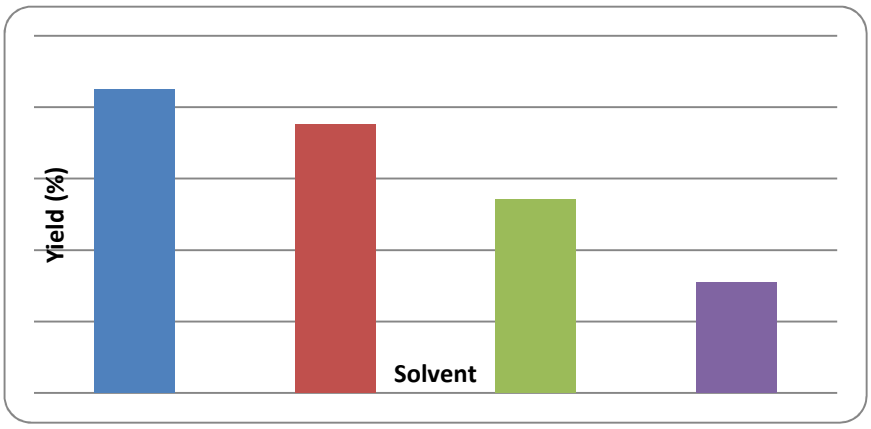

Figure 1. The extraction yields of *Vernonia amygdalina* using different solvent. ADE = aqueous extract obtained by decoction; AME = aqueous extract obtained by maceration; EE = extract obtained by maceration in ethanol; EAE = extract obtained by maceration in ethyl acetate.

Total phenolic content and total saponin content

The TP values (reported in mg gallic acid equivalents/ g dry extract) are shown in Table 1. The EE extract of *V. amygdalina* has the highest TP content. The TP contents of the extracts exhibited the descending order: EE > ADE > AME > EAE. The TP content of the EAE extract and the AME extract were not significantly different ($p > 0.05$) indicating that both the EAE and AME extracts have comparable amounts of phenolic compounds. The TP for EE, EAE and ADE however were significantly different ($p < 0.05$). Phenolic compounds are polar aromatic compounds that are readily soluble in ethanol[23] and the results shown in Table 1 confirms this as the EE extract of *V. amygdalina* has the highest TP value calculated. However, the presence of phenolic compounds in the ADE, AME and EAE extract could be attributed to the difference in the phenolic compound's structure and polarity.

Temperature also seems to play an important role in the extraction of phenolic compounds in plants as the ADE extract has a significantly higher TP content per gram crude extract as compared to the AME extract. This is because higher temperatures increases the solubility of the polyphenols and this in turn promotes a higher mass transfer rate.[24,25] Apart from that, higher temperatures also decreases the viscosity and surface tension of the solvent and this enables the solvent to penetrate the sample matrices which in turn improves the extraction yield of the targeted compounds.[24,25]

Total saponin content of the extracts is shown in Table1. The TS content of EAE extract was found to be the highest. The TS content of the extracts exhibited the descending order: EAE > EE > ADE > AME. The EAE and EE extracts showed significantly ($p < 0.05$) higher TS content when compared to the AME and ADE extracts. The TS content of ADE and AME were not significantly different ($p > 0.05$). The TS content between EE, EAE and ADE extract were significantly different ($p<0.05$). The higher TS content found in the EE and EAE extracts may be ascribed to the method, [14] since the vanillin reagent, which acts as a chromogen under acidic conditions, reacts with the aglycone portion of the saponin and not its sugar moiety. Therefore, sapogenins, which are the non-polar aglycone portion of the saponin[26] will also be quantified by the assay. The high TS content found in the EAE and EE extracts may indicate that most of the saponins found within the *V. amygdalina* leaves are sapogenin.

Table 1: Total phenolic contentand total saponin content of *Vernonia amygdalina* extracts

Extracts	Total Phenolics (mg GAE/g dry extract)	Total Saponin (mg DE/g dry extract)
EE	63.044 ± 2.602[a]	841.370 ± 22.455[a]
EAE	38.834 ± 1.594[b]	952.037 ± 28.701[b]
ADE	53.148 ± 0.799[c]	159.741 ± 4.207[c]
AME	39.391 ± 0.345 [b]	118.444 ± 19.699[c]

Mean ± SD. Means with the same letter are not significantly different from each other at p< 0.05 (ANOVA, followed by Tukey test). The values within the table were obtained by calculating the average
of three replicates ± standard deviation

In vitro free radical scavenging assay

Figure 2 shows DPPH radical scavenging activity of *V. amygdalina* extracts and the reference standard BHA. Table 2 shows the IC_{50} values of *V. amygdalina* extracts for DPPH and ABTS radical scavenging activity. The IC_{50} value of the AME extract is significantly higher than that of the ADE despite the fact that both of these extracts were prepared using water as the extraction solvent. A possible reason for this could be due to the higher TP content of the ADE as compared to the AME. The DPPH IC_{50} values of ADE, EE and EAE extracts are not significantly different ($p > 0.05$) despite the significant differences between the extracts' TP content. This would imply that there may be other compounds involved that could cause the reduction of the DPPH radical and this is supported by the data moderate correlation between the DPPH radical scavenging activity (IC_{50}) and TP contents

(Figure 4 a) of *V. amygdalina.* Venskutonis[27] reported that the hydroxyl group found in monosaccharides (which are water soluble) is capable of acting as a hydrogen donor and this causes the reduction of the DPPH radical from dark purple to yellow. This might explain the higher DPPH radical scavenging activity found in the ADE extract despite its lower TP value when compared to the EE extract. Similarly, saponins content which was found in high in the EAE extract (as shown in Table 1) may also contribute to the DPPH radical scavenging activity[28] since there was moderate correlation between the DPPH radical scavenging activity (IC_{50}) and TS contents of *V. amygdalina (*Figure 4 c).

In a study conducted by Ho *et al.*[9] using aqueous spray dried *V. amygdalin*a extracts, the IC_{50} value of the aqueous extract was reported to be 600 ± 7.4 µg/ml and this IC_{50} value is comparable to the IC_{50} value of the ADE extract obtained in this study. However, the IC_{50} values of the aqueous suspension of *V. amygdalina* extract reported by Adetutu*et al.*[8] was different. In Adetutu*et al.*'s study, 4.0 ± 0.1 µg/ml was the IC_{50} value reported for the DPPH radical scavenging activity. Comparable results were obtained between the study conducted by Ho *et al.*[9] and this study because both the plants used in the studies were obtained from the same geographical region (Malaysia) while the study conducted by Adetutu *et al.*[8] was done in Nigeria which may have a different geographical environment and thus resulting in a variation in chemical composition. For the EE extract, Ayoola *et al.*[29] reported an IC_{50} value of 2300 µg/ml and this value differs from the IC_{50} value obtained in this study due to the reasons mentioned above.

Figure 3 shows ABTS radical scavenging activity of *V. amygdalina* extracts and the reference standard Trolox. The IC_{50} values of the ABTS assay was different from the DPPH assay with the AME extract having a lower IC_{50} value when compared to the EE and EAE extracts (Table 2). The difference in radical scavenging activities between the ABTS and DPPH assays could be attributed to the stereo-selectivity of the generated radicals, the chemical structures of the compounds and the solubility of the *V. amygdalina* extracts in the test system used.[30, 31] Correlation of the TP and TS content against the ABTS radical scavenging activity (Figure 4b) revealed that the TP content had weak correlation with the observed ABTS radical scavenging activity. This indicates that the phenolic compounds in *V. amygdalina* are not responsible for the ABTS radical scavenging activity and that there may be other bioactive compounds that are responsible for the ABTS radical scavenging activity observed. The correlation between the TS and ABTS radical scavenging activity (Figure 4d) showed strong negative correlation as the ABTS radical scavenging activity decreases with increasing TS content. Currently, Li *et al.*[32], reported similar strong negative correlation between the TS and ABTS radical scavenging activity of *Rhizomacimicifugae*.

Table 2: IC_{50} values of *Veronica amygdalina* for DPPH and ABTS radical scavenging activity

Samples	**DPPH IC_{50} (µg/ml) ±SD**	**ABTS IC_{50} (µg/ml) ± SD**
EE	636.010 ± 59.325^{a}	5508.517 ± 411.998^{a}
EAE	658.277 ± 118.046^{ab}	6547.940 ± 368.270^{b}
ADE	501.207 ± 10.968^{ab}	3195.083 ± 435.890^{c}
AME	1368.929 ± 27.879^{c}	4142.156 ± 78.880^{d}
BHA	40.993 ± 1.317^{d}	-
Trolox	-	362.629 ± 13.354^{e}

Means with the same letter are not significantly different from each other at $p< 0.05$ (ANOVA, followed by Tukey test). The values within the table were obtained by calculating the average of three replicates ± standard deviation.

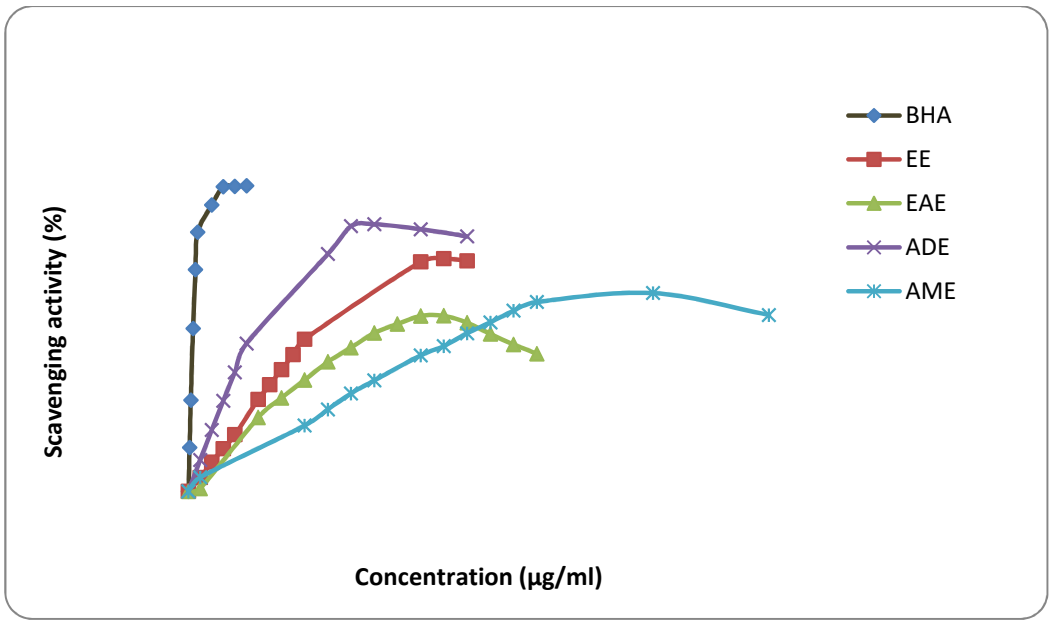

Figure 2: DPPH radical scavenging activity of *Vernonia amygdalina* extracts and the reference standard BHA.

Antimicrobial susceptibility test

The results of the antimicrobial screening are shown in Table 3. Of the four *V. amygdalina* extracts screened at 500 mg/ml, only the AME extract showed antimicrobial activity on *Y .enterocolitica* with an inhibition zone of 11.33 $\pm$ 0.58 mm. Therefore, the MIC was performed using only the aqueous maceration extract on *Y. enterocolitica.* The results of the MIC revealed that the AME extract had an MIC of 31.25 mg/ml against *Y. enterocolitica* while chloramphenicol had an MIC value of $\leq$ 8.000 x 10^{-3} mg/ml. Similar literature report by Owhe-Ureghe *et al.*[10] showed that the *Y. enterocolitica* O:8 strain was resistant to the ethanolic extract but susceptible to the water extract of *V. amygdalina*.

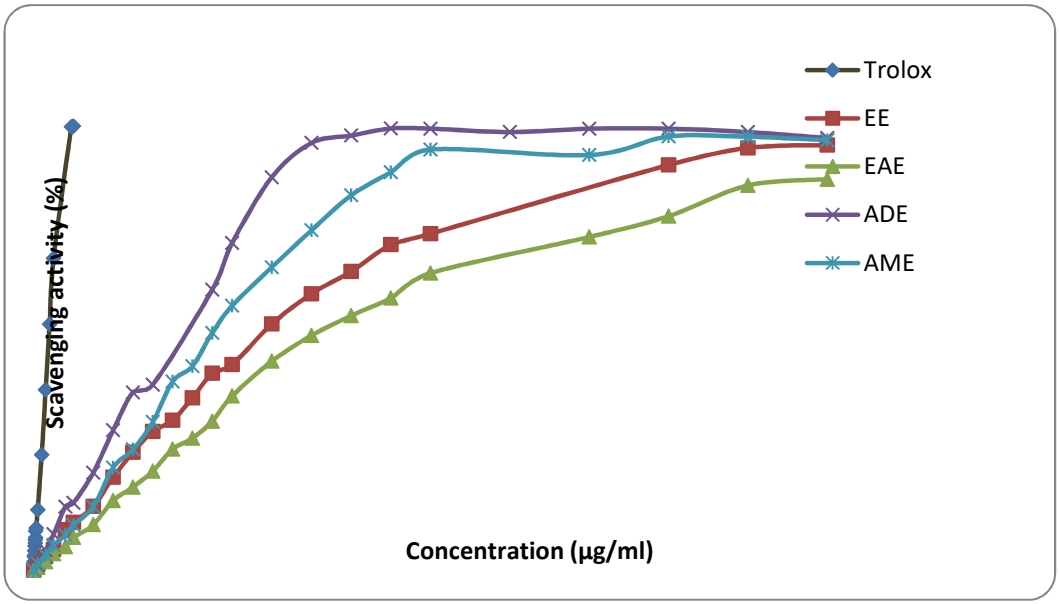

Figure 3: ABTS radical scavenging activity of *Vernonia amygdalina* extracts and the reference standard Trolox.

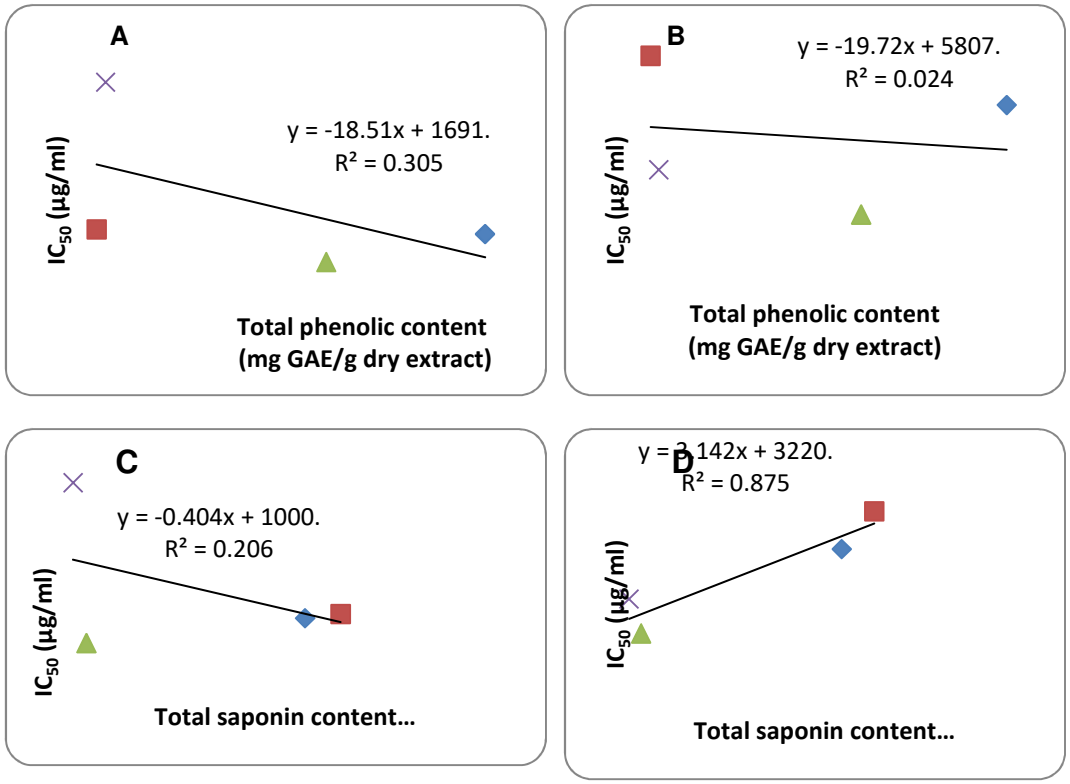

Figure 4: Correlation between (A) DPPH radical scavenging activity (IC_{50}) and total phenolic contents, (B) ABTS radical scavenging activity (IC_{50}) and total phenolic contents, (C) DPPH radical scavenging activity (IC_{50}) and total saponin contents, (D). ABTS radical scavenging activity (IC_{50}) and total saponin contents.

Table 3: Antimicrobial activity of *Vernoniaamygdalina* extracts (500 mg/ml) on selected bacteria

Extract	Inhibition zone diameter (mm)		
	***E. coli* O157:H7 (v)**	***E. coli* O157:H7 (c)**	***Y. enterocolitica***
EE	NI	NI	7.000 ± 0.000
EAE	NI	NI	7.500 ± 0.710
ADE	NI	NI	NI
AME	NI	NI	11.330 ± 0.580
Ampicillin	23.33 ± 0.580	24.33 ± 0.580	-
Chloramphenicol	-	-	29.330 ± 0.580
Water	NI	NI	NI
99.99% DMSO	NI	NI	8.000 ± 0.00

E. coli O157:H7 (v) = vegetable isolate; *E. coli* O157:H7 (c) = chicken isolate; NI = no inhibition; - = not applicable. The inhibition zone diameters shown were obtained by calculating the average of three replicates ± standard deviation.

The similarity in findings could be due to the same strains used as the *Y. enterocolitica* used in this study is also of the O:8 serotype. There have been reports of *V. amygdalina* having antimicrobial activity on other *E. coli* strains. [33] However, antimicrobial activity of *V. amygdalina* on *E. coli* O157:H7 has not been reported. The difference in susceptibility observed could be linked to the genetic diversity of the pathogen which gives rise to different resistant mechanisms.[34]

CONCLUSION

The choice of solvents and extraction method plays important role in the preparation of plant extracts. This study shows that the use of solvents of different polarity affects not only the extraction yield but also the content of the TS and TP; the free radical scavenging activities; the bioactive compounds that can be obtained and the antimicrobial activity of the extracts. The more polar solvents gave higher extraction yields than the less polar solvents. The EE extract showed the highest TP content and the EAE extract showed the highest TS content. This indicates that different bioactive compounds can be extracted with different solvents. The effect of temperature on the extraction yield, TP and free radical scavenging activities can also be seen when comparing the ADE and AME extract. The ADE extract exhibited higher extraction yield, TP content and better free radical scavenging activity than the AME extract. All the extracts demonstrated free radical scavenging activities with the ADE extract having the lowest IC_{50} value for both the DPPH and ABTS radical scavenging assay. This information helps to establish the potential of *V. amygdalina*as a source of dietary antioxidants. For the antimicrobial studies, both the *E. coli* O157: H7 isolates and *Y. enterocoitica*showed resistance against the *V. amygdalina* extracts tested. The antimicrobial studies provide information on the suitability of *V. amygdalina*against *E. coli* O157:H7 and *Y.enterocolitica*.

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CONSORTIUM