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Repression of some cholinergic and monoaminergic enzymes by non-polar solvent extracts from Rosary Pea (*Abrus precatorius*)

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ABSTRACT: This study focused on the effects of four different solvents (ethanol, acetone, dichloromethane (DCM) and N-hexane) extractions of *Abrus precatorius* leaves on neurological enzymes [acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and monoamine oxidase (MAO)], which are markers of Alzheimer's disease (AD), as well as the antioxidant [1,1-diphenyl-2 picrylhdrazyl] (DPPH) radical scavenging and ferric reducing antioxidant property (FRAP) potentials. The results revealed that acetone and N-hexane extracts had the highest AChE inhibitory effect having an EC_{50} value of 0.28 and 0.27 mg/ml, respectively, with no significant difference, while ethanol (EC_{50} = 0.61 mg/ml) extract had the least on BChE activity. Also, on MAO activity, acetone (0.24 mg/ml) and N-hexane (0.24 mg/ml) extracts had the highest inhibitory effect with no significant difference. The total phenol and flavonoid contents of the extracts ranged between 4.51 - 9.47 mg GAE/100 g and 0.03 - 0.41 mg QE/100 g respectively. All the extracts scavenged DPPH radical and possessed Fe reducing power. The inhibition of cholinesterases and MAO activities as well antioxidative potential of *A. precatorius* could be some of its possible mechanisms explore in folklore for the management of neurodegenerative diseases. However, *in vivo* and clinical studies should be carried out to ascertain these claims.

Keywords: *Abrus precatorius*, cholinesterases, neurodegeneration, antioxidants, solvent

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Introduction

In recent years, plants are commonly used as an alternative source to combat health problems in developing countries, because of the several side effects exhibited by synthetic drugs (Costa *et al.,* 2015). *Abrus precatorius* is a leguminous crop of the plant family Fabacea. It is a slender perennial climber that twines around trees, shrubs and hedges with no special organ of attachment. Leaves of *A. precatorius* are glabrous with long internodes, having a slender branches with cylindrical wrinkled stem (Hara and Williams, 1979; Fernando, 1988). *A. precatorius* is also known as rosary pea, precatory pea or bean, jequirity, crab's eye, John Crow bead, Indian licorice, Akar Saga, gideegidee or Jumbie bead in Trinidad and Tobago (Wagstaff, 2008). This plant is well known for its seeds, which are found in a variety of colours such as red, which is the most common variety with a glossy appearance with the black band at

the end that attaches to the plant and also black and orange seeds (Fernando, 2001). The seed is used as beads and in percussion instruments, and is known for its toxic nature due to the presence of the toxic principle, abrin (Bisby, 1994).

Management of neurodegenerative conditions, especially Alzheimer's disease (AD) has been with the use of drugs, such as cholinesterase inhibitors, consequently increasing brain's acetylcholine. This is because in AD conditions, there are elevated cholinesterase activities (Vladimir-Kneevic *et al.,* 2014), which function in degrading the neurotransmitters acetylcholine and butyrylcholine thereby giving rise to the symptoms observed in AD. Nevertheless, due to the apparent toxicity and cost encountered with the use of synthetic cholinesterase inhibitors, the focus is now on the use of natural sources of cholinesterase inhibitors as a remedy for this disease (Vladimir-Kneevic *et al.,* 2014). Monoamine oxidase (MAO) inhibitors have also been employed in the therapeutic measure towards alleviating Parkinson disease (Riederer *et al.,* 1989). Studies on some sea weeds (Yoon *et al.,* 2008), vegetables and herbs (Ferreira *et al.,* 2006; Mukherjee *et al.,* 2007) have shown the great therapeutic importance in cholinesterase inhibition. Oral consumption of water extract of dried *A precatorius* leaves and roots in Brazil has been reportedly served as a nerve tonic (Elisabetsky *et al.,* 1992). According to Prashant *et al*. (2011), method of extraction, solvent type, polarity as well as the concentration of the solvent, among others, does affect the extraction of phytochemical compounds. Hence, this study aims to determine the effects of different solvent extractions on the cholinesterases [acetylcholinesterase (AChE), butrylcholinesterase (BChE)], monoamine oxidase (MAO) and antioxidant properties of rosary pea (*Abrus precatorius*) leaves.

Materials and Methods

Sample Collection

Abrus precatorius leaves were collected from Ilogbo Ekiti, Southwest, Nigeria. The authentication of the plant was done in the Department of Crop and Pest Management, Federal University of Technology, Akure, Nigeria. The leaves were air dried for 7 days and ground into powder.

Preparation of extracts

The air-dried leaves of the plant were powdered with a laboratory grinder to obtain a powder, which was then subjected to extraction using different solvents (N-hexane, acetone, ethanol and dichoromethane) followed by shaking in an orbital shaker for 4 h at room temperature. The extracts were subjected to rotary evaporator to obtain a powder form which was reconstituted and centrifuged at 4000 RPM to obtain clear supernatant, which were then stored for subsequent analysis.

Chemicals and reagents

All chemicals used were purchased from Sigma Co. (St. Louis, MO). Unless stated otherwise, all chemicals used were of analytical grade and the water was glass distilled.

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibition assay

Inhibition of AChE was assessed by a modified colorimetric method of Perry *et al.,* 2000. The AChE activity was determined in a reaction mixture containing 200 µL of a solution of AChE (0.415 U/ml in 0.1 M phosphate buffer, pH 8.0), 100 µl of 5, 5'-dithiobis (2-nitrobenzoic) acid solution (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mM), the extracts (0 - 100 µl), and 50 µl phosphate buffer, pH 8.0. After incubation for 20 min at 25ºC, 100 µl of 0.05 mM acetylthiocholine iodide solution was added as the substrate, and AChE activity was determined as changes in absorbance reading at 412 nm for 3 min at 25°C using a spectrophotometer. 100 µl of butrylthiocholine iodide was used as a substrate to assay butyrylcholinesterase activity, while all other reagents and conditions were the same. The AChE and BChE inhibitory activities were expressed as percentage inhibition.

Monoamine oxidase (MAO) inhibition assay

The MAO inhibitory activity of the extracts was carried out according to Green and Haughton, (1961) and Turski et al. (1973) with slight modification. Briefly, the reaction mixture contained 25 mM phosphate buffer (pH 7), 12.5 mM semicarbazide, 10 mM benzylamine (pH adjusted to 7), tissue homogenate and appropriate dilutions of extracts in a total reaction volume of 2 ml. After 30 min, 1 ml of acetic acid was added and boiled for 3 min in boiling water bath followed by centrifugation. The resulting supernatant (1 ml) was mixed with equal volume of 0.05% of 2, 4-DNPH and 2.5 ml of benzene was added after 10 min incubation at room temperature. After separating the benzene layer it was mixed with equal volume of 0.1 M NaOH. Alkaline layer was decanted and heated at 80°C for 10 min. The orange-yellow colour developed was measured at 450 nm. The MAO inhibitory activity was expressed as percentage inhibition.

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.*, (1999). Briefly, appropriate dilutions of the extracts (200 µl) were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by the addition of 2.0 ml of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45ºC and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda *et al.*, (2005). Briefly 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl 10% $A1C1_3$, 50 µ1 of 1M potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30min. The absorbance of the reaction mixture was subsequently measured at 415 nm; the total flavonoid content was subsequently calculated.

1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhdrazyl) free radical was evaluated as described by Gyamfi *et al*. (1999). Briefly, appropriate dilution of the extracts (0 – 500 µl) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Determination of ferric reducing antioxidant power (FRAP)

The reducing power of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 500 µl aliquot of the extracts was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 801 × *g* for 10 minutes. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing power was subsequently calculated using ascorbic acid equivalent.

Data analysis

The results of the three replicates were pooled and expressed as a mean±standard deviation (S.D.). A Student's t-test, one-way analysis of variance (ANOVA) and least significance difference (LSD) were carried out. Significance was accepted at $p < 0.05$. EC_{50} was determined using linear regression analysis.

Results

The effect of different solvent extraction of *A. precatorius* on the activities of acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and monoamine oxidase (MAO) *in vitro* are presented in Figures 1, 2 and 3 respectively with their EC_{50} values in Table 1. The result revealed that all the extracts inhibited AChE in a dose dependent manner (0 - 0.58 mg/ml); however, N-hexane extract (IC_{50}= 0.27 mg/ml) had the significantly ($p < 0.05$) highest inhibition of AChE activity than other extracts while ethanol extract (IC_{50}= 0.45 mg/ml) had the least inhibition of AChE activity *in vitro*.

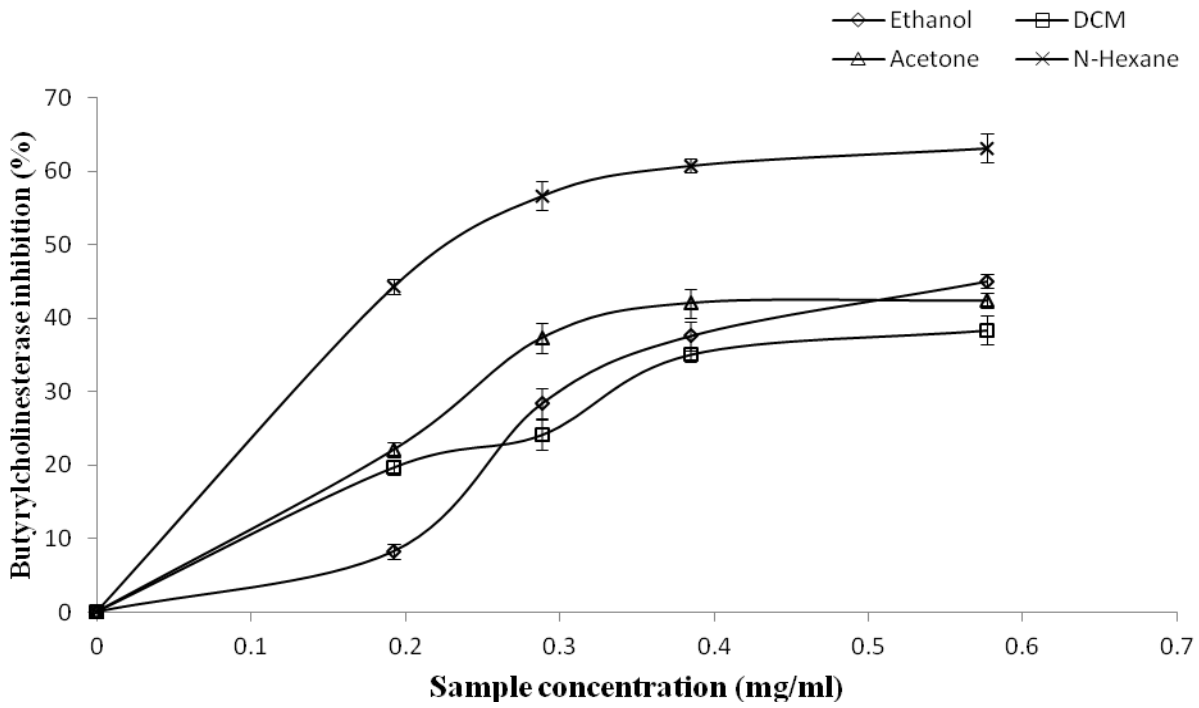

Figure 1. Butyrylcholinesterase inhibitory activity of different solvent extraction of Rosary pea leaf
Values represent mean ± standard deviation (n=3).

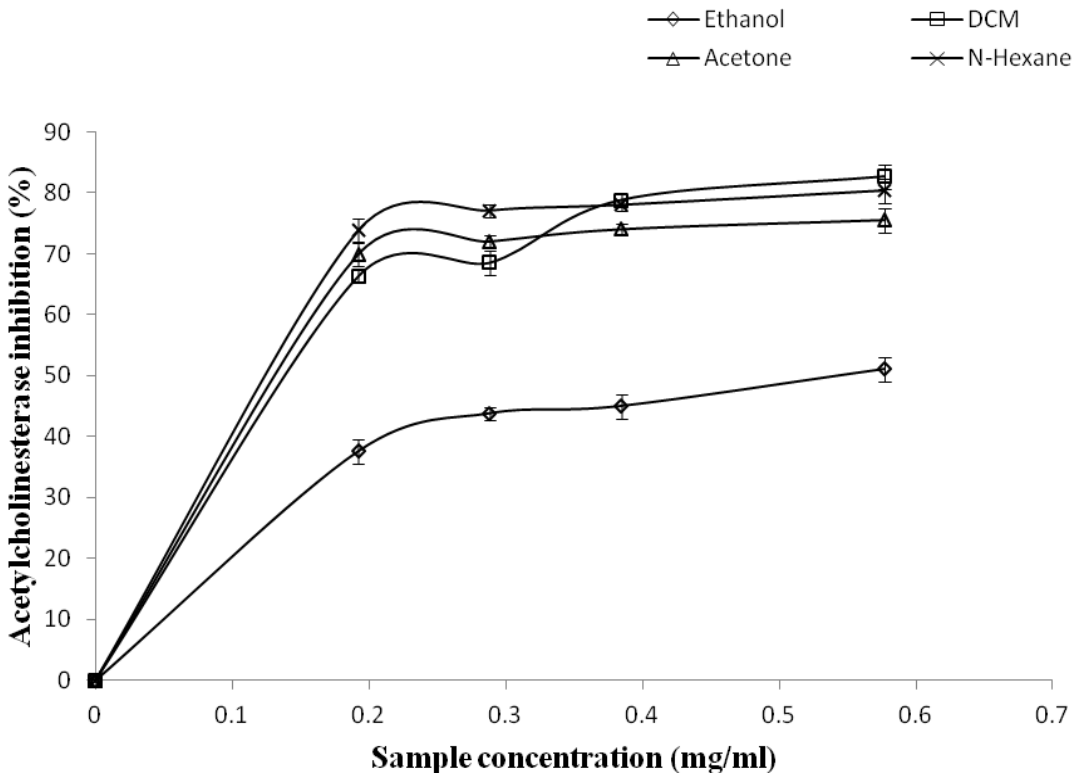

Figure 2. Acetylcholinesterase inhibitory activity of different solvent extraction of Rosary pea leaf
Values represent mean ± standard deviation (n=3).

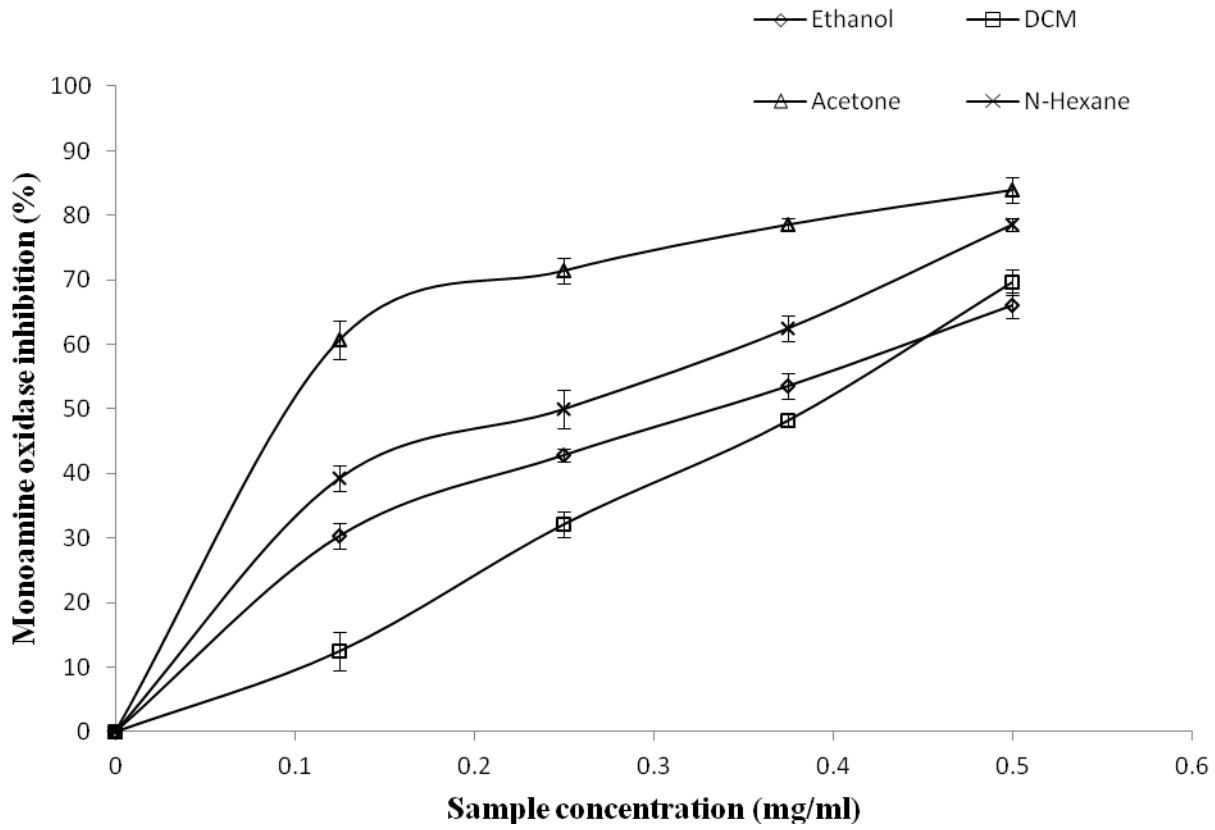

Figure 3. Monoamine Oxidase inhibitory activity of different solvent extraction of Rosary pea leaf

Values represent mean ± standard deviation (n=3).

Table 1: IC_{50} of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), Monoamine oxidase (MAO) and DPPH inhibitory activities of *Abrus precatorius* extracts.

Sample Extract	IC_{50} (mg/ml)			
	AChE	BChE	MAO	DPPH
Ethanol	$0.45 \pm 0.0.03^{a}$	$0.61 \pm 0.0.02^{a}$	$0.35 \pm 0.0.05^{a}$	$0.85 \pm 0.0.01^{a}$
DCM	$0.28 \pm 0.0.01^{b}$	$0.65 \pm 0.0.01^{a}$	$0.38 \pm 0.0.03^{a}$	$0.81 \pm 0.0.01^{a}$
Acetone	$0.28 \pm 0.0.03^{b}$	$0.54 \pm 0.0.02^{b}$	$0.24 \pm 0.0.02^{b}$	$0.82 \pm 0.0.02^{a}$
N-Hexane	$0.27 \pm 0.0.02^{b}$	$0.36 \pm 0.0.02^{c}$	$0.29 \pm 0.0.02^{ab}$	$0.76 \pm 0.0.01^{b}$

Values represent mean ± standard deviation (n=3). Values with the same superscript along the same column are not significantly different ($p<0.05$).

The result also revealed that all the extracts inhibited BChE in a dose dependent manner (0 - 0.58 mg/ml); however, N-hexane extract (IC_{50}= 0.36 mg/ml) had the significantly ($p<0.05$) highest inhibition of BChE activity than other extracts while DCM extract (IC_{50}= 0.65 mg/ml) had the least inhibition of BChE activity *in vitro*. The result of MAO inhibition revealed that all the extracts inhibited MAO in a dose dependent manner (0 - 0.5 mg/ml); however, N-hexane extract (IC_{50}= 0.29 mg/ml) had the significantly ($p<0.05$) highest inhibition of MAO activity than other extracts while DCM extract (IC_{50} = 0.38 mg/ml) had the least inhibition of MAO activity *in vitro*. Furthermore, the DPPH* radical scavenging ability of *A. precatorius* extracts revealed that all the solvent extraction of *Abrus precatorius* scavenged DPPH* radical in a dose-dependent manner (0-1.67 mg/ml) with N-Hexane extract (IC_{50} = 0.76 mg/ml) having the highest significantly ($p<0.05$) DPPH* scavenging ability and ethanol extract (IC_{50} = 0.85 mg/ml) having the least scavenging ability.

The results of the total phenol, total flavonoid and ferric reducing antioxidant property (FRAP) of the extracts of *Abrus precatorius* are shown in Table 1 respectively. The result revealed that acetone extract (9.47 mg GAE/100g) had significantly ($p<0.05$) higher total phenol content than other solvent extracts with ethanol extract (4.51 mg GAE/100g) having the least total phenol content. The result also revealed that acetone extract (0.4 mg QE/100g) had significantly ($p<0.05$) higher total flavonoid content than other extracts with ethanol extract (0.03 mgQE/100 g) having the least total flavonoid content. However, the FRAP result revealed that the extracts were able to reduce Fe^{2+} with the DCM extract (9.8 mg AAE/g) having higher FRAP than other extracts with the least observed with the ethanol extract (7.4 mg AAE/g).

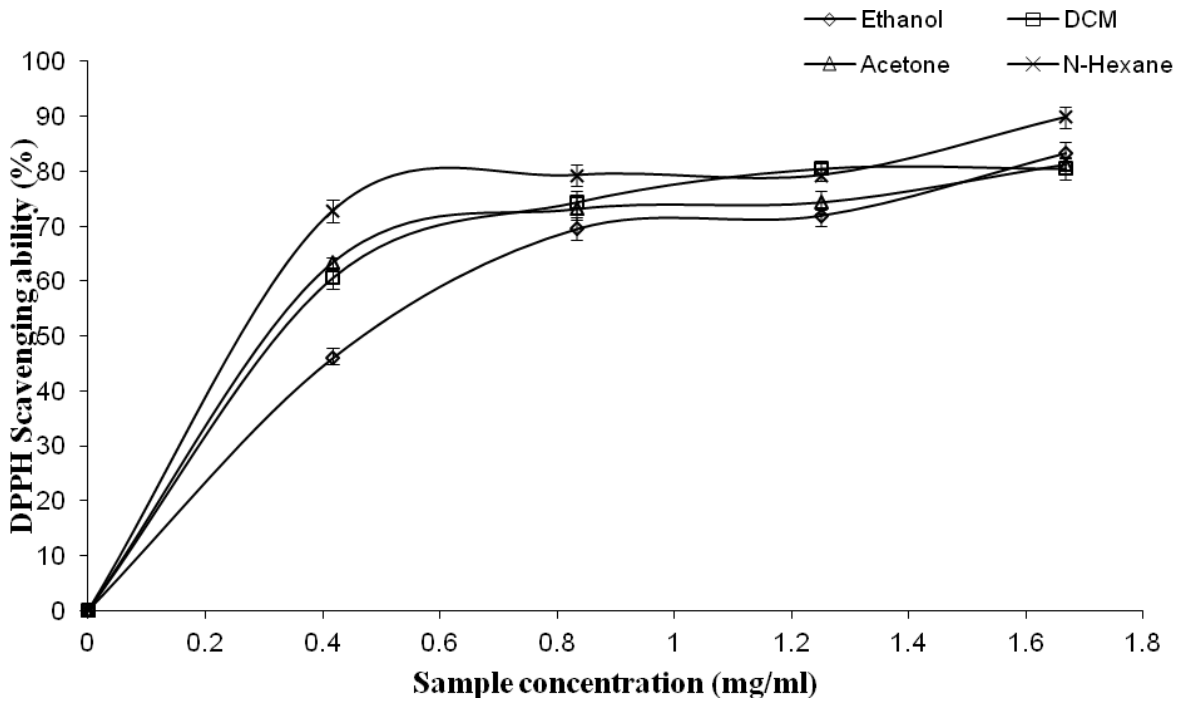

Figure 4. DPPH Radical Scavenging activity of different solvent extraction of Rosary pea leaf
Values represent mean ± standard deviation (n=3).

Table 2: The total phenol (mg GAE/100g), total flavonoid (mg QE /100g) and Ferric reducing antioxidant property (FRAP) (mg AEE/g) of *Abrus precatorius* extracts.

Samples	Total phenol (mg GAE/100g)	Total flavonoid mg QE/100g)	Reducing power (mg AEE/g)
Ethanol	4.51 ± 0.02^{c}	0.03 ± 0.01^{c}	7.42 ± 0.03^{b}
DCM	7.62 ± 0.06^{b}	0.23 ± 0.01^{b}	9.81 ± 0.03^{a}
Acetone	9.47 ± 0.01^{a}	0.41 ± 0.02^{a}	7.81 ± 0.02^{b}
N-Hexane	5.25 ± 0.02^{c}	0.04 ± 0.01^{c}	8.33 ± 0.01^{ab}

Values represent mean ± standard deviation (n=3). Values with the same superscript along the same column are not significantly different ($p<0.05$).

Discussion

Different clinical reports have demonstrated the chemopreventive ability of plant-derived phenolic compounds to be inexpensive, accessible, readily available and acceptable approach to different disease control and management (Huang *et al.,* 2010). The inhibitory effect of different solvent extraction of *A. precatorius* leaves on some key enzymes linked to neurodegeneration [acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and monoamine oxidase (MAO)] *in vitro* used in this study suggests their potential usefulness in the management of neurodegenerative conditions. The result revealed that all the extracts inhibited AChE, BChE and MAO activities in a dose dependent manner. However, the N-hexane extract of the *A. precatorius* leaf had the highest inhibitory effects on AChE and BChE activities while acetone extract had the highest on MAO activity. The inhibition of AChE and BChE activities of the extracts agreed with earlier reports on several plants showing cholinesterase inhibitory activity, thereby making them relevant in the treatment of neurodegenerative disorders (Das *et al.,* 2002; Perry *et al.,* 2001). Likewise, the observed inhibition of MAO activity by various extracts of *A. precatorius* leaves is in accordance with earlier reports on MAO inhibitors being shown to be effective in the management and/or treatment of neurological disorders (Youdim *et al.,* 2006)

Several studies reported that AD brain is under intense oxidative stress (Ademosun and Oboh, 2014) and decrease in the cholinergic neurons has been shown to promote the amyloid protein deposition in the AD brain, which in turn favour amyloid protein-associated oxidative stress and neurotoxicity (Butterfield and Lauderback, 2002). The inhibition of AChE, BChE and MAO by the extracts of *A. precatorius* leaf is suggestive of their neuroprotective potentials. Medicinal plants such as *Abrus precatorius* have been used in folklore medicine for the management of neurodegenerative diseases, though there is little or no information on its possible therapeutic mechanisms. The result of this study revealed that all the extracts had a considerable amount of total phenolic content, however the acetone leaf extract had the significantly ($p<0.05$) highest total phenol content than other extracts with same trend noticed for the total flavonoid content. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al.,* 1996). Natural polyphenols are capable of removing free radicals, chelate metal catalysts, activating antioxidant enzymes, reducing α- tocopherol radicals, and inhibiting oxidases (Amic *et al.* 2003; Alia *et al.* 2003).

Oxidative stress has been a major factor linked with the pathogenesis and progression of neurodegenerative diseases such as AD. The reducing powers of the extracts [ethanol, dicholoromethane (DCM), Acetone and N-hexane) of *A. precatorius* were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} and the results are presented in Table 4.1 as ascorbic acid equivalents. As revealed by the results, all the extracts were able to reduce Fe^{3+} to Fe^{2+}, however, DCM extract (9.81 mg AEE/g) had the significantly ($p<0.05$) highest reducing property than other extracts. Reducing power is a novel

antioxidant defence mechanism; the two mechanisms available to affect this property are by electron transfer and by hydrogen atom transfer (Dastmalchi *et al.,* 2007). This is because the ferric-to-ferrous iron reduction occurs rapidly with all registrants, with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants (Halvorsen *et al.,* 2002).

Antioxidant capacities of the various solvent extracts of *A. precatorius* as typified by their DPPH* radical scavenging and Fe^{2+} chelating abilities were assessed and as revealed by the results, all the extracts scavenged DPPH* radical dose dependently. However, the N-Hexane extract exhibited the strongest DPPH* radical scavenging ability than other extracts. An important mechanism of antioxidant activity is the ability to collate and deactivate the transition metals such as iron, which is capable of generating free radicals from Fenton reactions. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al.,* 1996). DPPH radical is used as a stable free radical donor to determine the antioxidant activity of natural compounds and the scavenging of stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants (Suhaj, 2006; Ozturk *et al.,* 2007; Maizura *et al.,* 2011).

Conclusion

The inhibition of AChE, BChE and MAO with their antioxidant properties of different solvent extractions of *Abrus precatorius* make them a good source for the management of neurodegenerative conditions. Nevertheless, *in vivo* experiments and clinical trials are recommended.

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