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Anticholinesterase and antioxidants potentials of Abrus precatorius Seed and Leaf

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Abstract
Neurodegenerative disease is a term applied to a variety of conditions arising from a chronic breakdown and deterioration of the neuronswhich is associated with oxidative stress. Abrusprecatorius are used for therapeutic purposes because they contain several active substances that exhibit antioxidant properties.Thein-vitro anticholinesterase and antioxidant properties of the methanol extract of the leaves and seeds of Abrusprecatoriususing two types of extraction techniques; soxhlet and cold maceration was investigated in this study. The anticholinesterase property was evaluated using Ellman's method while antioxidant property was determined by evaluating the antioxidant activity (1,1-diphenyl-2-picrylhydrazyl (DPPH), radical scavenging activity, ferric reducing antioxidant power (FRAP), iron chelating activity and molybdate ion reduction assays), total flavonoid (by Aluminium chloride assay) and total phenolic content (by Folin-Ciocalteu assay).Seed extracts exhibited higheranticholinesterase activity, FRAP, iron chelating activity,total phenolic content and total antioxidant capacitythan the leaf extracts. The seed soxhlet extract have the highest activity and the leaf cold extract have the lowest activity. Higher total flavonoid content was obtained for the leaf extracts compared to the seed extracts, with leaf soxhlet extract exhibiting the highest activity and seed cold extract exhibiting the lowest activity. DPPH activity was highest for the seed extracts than the leaf extract, however highest activitywas observed in the seed cold extract while the lowest activity was observed in leaf cold extract.
In conclusion, seed extract of Abusprecatirius have more anticholinesterase and antioxidant than the leaf extract. These properties is affected by the method of extraction.
Key Word: *Anticholinesterase, Antioxidant, Abrusprecatorius, Neurodegenerative*

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I. Introduction

Free radicals generated in aerobic metabolism are involved in a series of regulatory processes such as cell proliferation, apoptosis, and gene expression. When generated in excess, free radicals can counteract the defense capability of the antioxidant system, resulting in oxidative stress. Oxidative stress results in the production of cytotoxic compound (malonyl dialdehyde, 4-hydroxynonenal) thereby altering the oxidant-antioxidant balance that characterizes normal cell functioning[1].Oxidative stress-induced pathology includes cancer[2], cardiovascular disease[3], neural disorders[4], Alzheimer's disease[5], mild cognitive impairment[6], Parkinson's disease[7], atherosclerosis[8], and aging[9]. Natural antioxidants constitute the essential part in the cell's defense mechanisms against these diseased states and they can be endogenous or exogenous. Plant contains several compounds such as vitamins (vitamin E and C), polyphenols, including flavonoids which possess antioxidant activities[10].Thus, consumption of herbs and spices has been beneficial in the prevention of many diseased state[11]. Increased antioxidant capacity and reduced oxidative stress are key mechanisms underlying the beneficial effects of plants.

Many evidences show that oxidative stress affects cognitive functions and contributes to the development of many neuropsychiatric and neurodegenerative disorders including depression, anxiety, Alzheimer's disease, and Parkinson's disease[12].

Dementia is a mental chronic disorder characterized by the loss of intellectual ability that results in impairment of memory. Alzheimer's disease (AD), which is a progressive neurodegenerative disorder is the most common cause of dementia[13]. Classical features of Alzheimer's disease and dementia includeneuronal loss in regions of the brain associated with memory and cognition, especially the cholinergic neurons. Neurotransmitter depletion mainly acetylcholine(ACh) and synaptic dysfunction may also occur[14].

Central cholinergic(acetylcholine-producing)system is considered as the most important neurotransmitter system involved in the regulation of cognitive functions. In the peripheral nervous system, acetylcholine plays a role in skeletal muscle movement, as well as in the regulation of smooth muscle and cardiac muscle.In the central nervous system, acetylcholine is believed to be involved in learning, memory, and mood[15]. Damage to the cholinergic system in the brain has been shown to be associated with the memory deficits associated with Alzheimer's disease[16].Cholinesterases (AChE) catalyses the hydrolysis of the neurotransmitter ACh. Cholinesterase inhibitors prevent the hydrolysis of the neurotransmitter acetylcholine by AChE and therefore improve cognitive function by reversing the cholinergic deficit[17].

Abrusprecatorius is a shrub that is commonly used in different part of West Africa for the treatment of several ailments which includes pain, fever and inflammatory conditions. The plant is best known for its seeds, which are used as beads and in percussion instruments, but which are toxic because of the presence of abrin[18,19]. The plant is native to India and grows in tropical and subtropical areas of the world where it has been introduced[20]. *Abrusprecatorius*belongs to the family Fabaceae, genusAbrus, species*Abrusprecatorius* and*subsp. africanus*. Seeds are poisonous and therefore are used after mitigation[21], which is done by many methods such as boiling the seeds in milk and then drying them. The protein, abrin is denatured when subjected to high temperature and thus the toxicity of the seed is removed. *Abrusprecatorius*are rich in several essential amino acids such as serine and it contain a number of constituents like alkaloids, flavonoids, tannins, triterpenoids[22,23,24]. Methanol and chloroform extracts of *Abrusprecatorius* posssess antibiotic properties against many bacteria such as, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli*[25]. Sagar *et al.,* 2014 have also shown that the seeds exhibit anti-inflammatory activity[26]. *Abrusprecatorius* plant exhibit anti-convulsant, anti-epileptic, immune-modulating, abortifacient[27], memory enhancing[28] and anti-serotonin[29]activities e.t.c.

The inhibition of acetylcholinesterase and oxidation are important in the development of drug for AD, but synthetic drugs such as BHT and BHA for reducing the effect of oxidation[30] and those for the treatment of AD (AChE inhibitors such as tacrine, and rivastigmine) have many side effects[31]. This created the need for a natural drug which possesses these two properties and this can be met in plants. Thus, the objective of this study was to evaluate the antioxidant and neuroprotective potential of *Abrusprecatorius* seedand leaf to treat the AD and other neurodegenerative disease. Furthermoreto compare the activity of the seed with the leaf and the effect of different extraction methods (cold maceration and extraction by heating i.esoxhletextraction) on the activities of the seed and leaf, since the seed is toxic and detoxification shouldbe performed with or without the help of heat before use and moreover, local herbs are usually prepared either by cold merceration or by boiling.

II. Materials and Methods

The plant materials, *Abrusprecatorius* leaf and seed were obtained from Ijebu-ode in Ogun state, Nigeria.The samples were identified by an expert botanist at the forestry research institute, Nigeria.

The fresh leaves and seeds of *Abrusprecatorius* were washed, air dried for eight weeks andpowdered. The methanol extracts of the leaves and seeds were obtained using cold maceration and soxhlet extraction. 10g of the dried powder of *Abrusprecatorius* leaves and seeds were macerated in 50ml of 80% methanol at 4°C for 24 hours using a magnetic stirrer. The filtrate was obtained by passing the mixture through Whatman No. 1 filter paper.10g each of dried powder was also extracted with 50ml of 80% methanol using the soxhletapparatus and the resulting extracts were concentrated using rotary evaporator at 45^0C. Dried samples were obtained by exposing the concentrates to air at room temperature.

REAGENTS

Methanol, ammonium molybdate,, ferric chloride, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2,4,6-tris(2-ptridyl)3-triazine (TPTZ), hydrochloric acid, distilled water, sodium phosphate, hydrogen tetraoxosulphate (vi) , sodium carbonate, Folin-Ciocalteu's phenolic reagent,, aluminium chloride, sodium hydroxide, EDTA, ferrozin, iron(ii)tetraoxosulphate(vi), sodium acetate buffer, sodium nitrite, quercetin, gallic acid, ascorbic acid, tris buffer, 5,5-dithiobis[2-nitrobenzoic acid] (DTNB), Acetylthiocholine iodide (ATChI), brain homogenate of rat (for ACHE acivity) were purchased fromSigma-Aldrich Chemical, United Kingdom

DDPH ASSAY

The radical scavenging ability of the extract was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described by Brand-Williams and others[32]. The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to the reduction of DPPH[33]. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517nm.150µl of the each of the extracts and standard were pipetted into the microplate in triplicate for seven different concentrations. To these was added 150µl of DPPH solution in the micro plate and incubated in the dark for 30minutes. Absorbance of solutions were taken at 517nm using the UV-visible spectrophotometer micron plate reader. L-Ascorbic acid was used as

the reference compound. The antioxidant activity is expressed in terms of IC_{50} (concentration of the extract or standard compound required to inhibit DPPH radical formation by 50%). The percentage inhibition was calculated using a formula:

$\{(A_C - A_0)/ Ac\}x\ 100$

Where Ac = absorbance of negative control and A_0= absorbance of sample.

Total phenolic content

Total phenolic content was analyzed by the oxidizing effect of Folin–Ciocalteuphenol reagent, based on the procedure of Singleton and Rossi (1965)[34] and as reported by Gulcin*et al.*[35]. To 1ml of 0.1mg/ml of the extract and varying concentrations of standard solutions of gallic acid was added 200µl of folin-ciocalteu's reagent in different test-tubes. After 5minutes 1ml of 7% sodium carbonate and 0.3ml of distilled water was added to all tubes. The mixtures were incubated at room temperature for 90 minutes and absorbance was measured at 750nm. Samples were analyzed in triplicates; averages of the results were determined and expressed as mg gallic acid equivalent per gram dry weight extract (mg GAE/g extract).

Determination of total flavonoid contents

Total flavonoid content was measured by the Aluminium chloride colorimetric assay as described by Neergheen *et al.*, [36]. 25µl of $NaNO_2$ and 100µlof distilled water was added to 25 µL of 1mg/mL extract solution and to 25µl of varying of the quercetin standard in a micro plate in triplicates and incubated at room temperature for 5minutes. Thereafter 25µl of $AlCl_3$ was added into each of the mixtures, with immediate addition of 50µl NaOH. Finally, 25µL of distilled water was added to make an overall volume of 0.25 ml. All reagents were freshly prepared. The absorbance was measured at 510nm. Averages of the results were determined and total flavonoids content was calculated as quercetin equivalent (mg QE/g extract).

Ferric reducing antioxidant power (frap) assay

The FRAP assay measures the reducing power of antioxidant in a redox linked colorimetric method of Benzie and Strain[37], which is based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous coloured form.25µl of the extracts (1mg/mL), and ascorbic acid (standard) at six different concentrations in triplicate were mixed individually in the micro platewith300µL of FRAP reagent containing 300 mM Acetate buffer, 0.003g of TPTZ (2,4,6-Tris(2-pyridyl) 3-triazine) in 1 ml of 40 mM HCl and 18.6µl of 20 mM ferric chloride in 5ml of water in ratio 10:1:1.Each mixture was incubated at 37oC for 90 minutes and absorbance was measured at 593 nm. The higher absorbance of the reaction mixture indicates strong reducing power of the plant extract.

Total antioxidants capacity (TAC)

This method is based on the reduction of molybdenum (VI) to molybdenum (v) by the extracts and subsequent formation of a green phosphate molybdenum (v) complex at an acid pH[38]. To 25µl of the extracts (1mg/mL) and 25µl of the varying concentrations of standard was added 300µl of the TAC reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate)in the micro plate. Mixtures were prepared in triplicate and incubated at 95^0C in a water bath for 90minutes. Absorbance was read at 630nm.The results were expressed as mg ascorbic acid equivalent per gram dry weight extract (AAE)/g extract.

METAL CHELATING ASSAY

The method of Singh and Rajini, 2004 was adopted for the determination of the ferrous ion-chelating (FIC) activity of the extracts[39]. The test is based on the capacity to decolorize the iron-ferrozine coloured complex formed by the action of Ferrozine on iron. 100µl of varying concentrations of extractsand standard (EDTA) was mixed with 100µl of 0.1 mM $FeCl_2 \cdot 4H_2O$. After 5 minutes incubation at room temperature, the reaction was initiated by the addition of 100µl of 0.25mM ferrozine. The mixture was shaken vigorously and incubatedfurther at room temperature for 10 minutes. The absorbance of the solution was measured spectrophotometrically at 562nm.

The percentage inhibition of ferrozine–Fe^{+2} complex formation was calculated by using the formula:

Chelating effect % = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ = absorbance of control sample and A_{sample} = absorbance of a tested samples.

Anti-cholinesterase assay

The determination of acetylcholinesterase (ACHe) was carried out according to the method described by[40],modified by[41]. The source of the enzyme (acetycholinesterase) was the brain homogenate of a rat obtained by homogenizing the brain in 5 volumes of 10 mMTris-HCl buffer (pH 7.2), which contained 1 M NaCl, 50 mM $MgCl_2$ and 1 % Triton X-100. The homogenate was centrifuged at 10,000 *g* for 30 minutes at 4 °C and the

resulting supernatant, diluted 5 times, was used as an enzyme source. 240µl of 50 mM sodium phosphate buffer (pH 8.0)was added to 20µl of varying concentration of the extracts and standard inthe micro plate in triplicate. After addition of 20µl of the enzyme, the mixtures were incubated for 30 minutes at 37^0C. 20µl of 10Mm DTNB was then added and the reaction was initiated by addition of 20µl of 25mM ATChI (substrate). The absorbance was read at 412nm over a period of 4 minutes at 30 seconds interval.
The percentage inhibition of the extracts was obtained using the formula:
$\{(V_0 – V_i) / V_0\}$ x 100; where V_i= enzyme activity in the presence of extract and V_0 = enzyme activity in the absence of extract.

III. Results

Table 1: DPPH radical scavenging activity of *A. precatorius* leaf, seed and standard

Extract	IC_{50} ± S.D (mg/ml)
STANDARD	0.006635± 0.000194
Leaf cold maceration	0.578892 ± 0.023058a
Leaf soxhlet method	0.08304 ± 0.000517ab
Seed cold maceration	0.002267 ± 0.000661bc
Seed soxhlet method	0.012227 ± 0.000117bc

TABLE 2: Ferric reducing activating power of A. precatorius.

Extracts concentration (mg/ml)	Mean mg ascorbic acid Eq/g ± SD
Leaf cold maceration	9.055299 ± 1.143075
Leaf soxhlet method	31.97814 ± 1.774469
Seed cold maceration	260.2234 ± 19.32325ab
Seed soxhlet method	342.756 ± 5.566811abc

TABLE 3: Total Antioxidant Capacity of A. precatorius

Extracts Concentration (mg/ml)	Mean mg ascorbic acid Eq/g ± SD
Leaf soxhlet method	127.43255 ± 16.55758
Leaf cold maceration	94.97± 16.37049
Seed soxhlet method	536.35 ± 43.96808 ab
Seed cold maceration	271.4125 ± 20.54046abc

Table 4 : Metal chelating assay of A. precatorius leaf, seed and standard

Extract	**IC_{50} ± SD (mg/ml)**
Standard	0.063815 ± 0.014651
Leaf soxhlet method	1.291527429 ± 0.033465182a

Leaf cold maceration	4.040267 ± 0.078901ab
Seed soxhlet method	0.959425 ± 0.079361abc
Seed cold maceration	1.063755 ± 0.24672ac

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TABLE 5: Total phenolic content of A. precatorius.

Extract Concentration (mg/ml)	Mean mg Gallic acid Eq/g ± SD
Leaf cold maceration (1mg/ml)Seed	31.10293 ± 0.876962
Leaf soxhlet method (1mg/ml)	44.06248 ± 3.751449
Seed cold maceration (1mg/ml)	175.8991 ± 2.289846ab
Soxhlet method (1mg/ml)	238.943 ± 9.938905abc

TABLE 6: Total flavonoid content of A. precatorius

EXTRACTS Concentration (mg/ml)	Mean (mgQUE/g) ± SD
Leaf cold maceration (1mg/ml)	157.2195 ± 13.3002
Leaf soxhlet method (1mg/ml)	206.9823 ± 14.08638 a
Seed cold maceration (1mg/ml)	81.79768 ± 14.58653ab
Seed soxhlet method (1mg/ml)	139.336 ± 16.13793 bc

Table 7: Acetylcholinesterase activity assay of A. precatorius leaf and seed.

Extracts	IC_{50} ± SD (mg/ml)
Leaf soxhlet method (1mg/ml)	7. 3929 ± 0.3793
Leaf cold maceration	9.052707 ± 1.600707
Seed soxhlet method	2.823005 ± 0.899431abc
Seed cold maceration	5.392917 ± 0.33837a

IV. Discussion

Medicinal plants are rich in secondary metabolites such as polyphenols and flavonoids, and this account for their therapeutic effects. Oxidative stress which is an imbalance between the production of reactive oxygen species and level of antioxidant in the body system have been implicated in the pathogenesis of various diseases such as Alzheimer's disease,thus, the use of medicinal plants for the treatment and prevention of these diseases can be attributed to their rich source of antioxidant[42].The mechanism by which Antioxidants act include preventing the propagation of oxidative chain reactions, scavenging free radicals, regulating gene expression, taken part in the redox reaction and preventing free radical formations[43]. Some of these properties were exploited in the various assays for evaluation of the antioxidant properties of *A. precatorius.* Antioxidant properties of the leaves and seeds of *A. precatorius* were determined by FRAP, total antioxidant capacity and total phenolic content which involves the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls, metal ion-chelating activity prevents the generation of free radical, DPPH radical scavenging activity which measures the ability of the extracts to scavenge free radicals and the flavonoid content was also determined.

Invitro antioxidant study of *A. precatorius* seeds was performed using the cold methanol and soxhletextractextract of the leaves and seeds.

DPPH radical scavenging activity

In this study, Seeds Cold extract was found to exhibit the highest the DPPH radical scavenging activity among all four extracts with $IC_{50}$of 0.0023±0.0007mg/ml. This activity was also higher than that of the standard ascorbic acid (0.0066 ± 0.00019mg/ml), while the others exhibited lower activities (in increasing order) than that of the standard ascorbic acid, with IC_{50} values as follows; leaves cold extract (LCE) (0.579±0.023mg/ml), leaf soxhlet extract (LSE) (0.083±0.012mg/ml) and seeds soxhlet extract (SSE) (0.012±0.00012mg/ml) (Figure). Similar results were obtained by [44], who reported that the methanol extract of *A. precatorius* seeds exhibited the highest DPPH radical scavenging activity with respect to other parts of the plant, Rashmi *et al.,* reported that ethanol extract of *A. precatorius* seeds showed comparable DPPH radical scavenging activity with the standard ascorbic acid[45], while Mohammad Madaki*et al.,* indicated that the leaves possess lower DPPH radical scavenging activity than standard ascorbic acid[46]. $DPPH^{\cdot}$ assay expresses the radical scavenging activity of extracts and its results is a measure of phenolic and flavonoid compounds in seeds and leaves extracts[47]. However, there is discrepancy in the antioxidant activity values in DPPH (as expressed in the results above) in relations to the phenolic and flavonoid content as well as the antioxidant activities of the other assays (FRAP, TAC, metal chelating activity) for the extracts, as the sohxelet extracts exhibited the highest values in terms of other antioxidant assays and in terms of the phenolic and flavonoid contents. This may be as a result of different compounds acting as antioxidants through different mechanisms. Therefore, recommendation has been made that at least three different assays should be considered in assessing the antioxidant activity of plant extracts[48]. Thus the phenolic compounds present in the cold extract may be more active in scavenging free radicals.

Ferric Reducing Power Assay (FRAP)

The seed extracts (SSE and SCE) showed higher FRAP values (342.76± 5.566811mg AAE/g and 260.22± 19.32325mg AAE/g of sample respectively) than those obtained for leave extracts (LCE = 20.05 ± 1.143075mgAAE/g) and (LSE= 31.98mg ± 1.774469 AAE/g),with the SSE exhibiting the highest activity and the LCE exhibiting the lowest activity(figure). Vanitha *et al.,* reported that the leaves of showed high ferric reducing power at a lower concentration[49] while Marimuthu *et al.,* revealed that the ferric ferric reducing power activity of the seeds was higher than that of the leaves[50].The ferric reducing power of a compound is a reflection ofits ability to transfer electron and thusindicates its potential antioxidant activity[49]. This is reflected in the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. This suggests that SSEmight contain highest amounts of reductone; may possess the highest ability to react with free radicals to alter them into more stable non-reactive species and thus terminate radical chain reaction.

Total Antioxidant Capacity (TAC)

TAC for SSE, SCE, LSE and LCE is in decreasing order is as follows; 536.35 ± 43.96808 mg AAE/g, 271.41 ± 28.57099 mg AAE/g, 127.43 ± 20.54046 mg AAE/g, 94.97± 16.37049 mg AAE/g respectively. This is in line with the results for FRAP above.Earlier authors have also shown that the leaves and seedsextracts possess total antioxidant capacity[50, 51]. The phosphomolybdate method for determination of total antioxidant capacity measures the electron donating capacity which is an indicator of antioxidant activity[52], thus the seeds extract possesses higher electron donating capacity than the leaf extract, and soxhlet extraction in both cases exhibited a better electron donating capacity.

Metal Chelating Activity

The extracts exhibited metal chelating activities lower than the standard EDTA value in increasing other with IC_{50} values as follows; LCE, LSE, SCE and SSE with valuesof 6.5943 ± 0.2794, 4.0403 ± 0.0789, 1.2915 ± 0.0335, 1.0638 ± 0.2467and 0.959425 ± 0.079361 respectively. The results follow the same trend as FRAP and TAC.The ability of antioxidant to chelate ferrous iron reduces free radical reactions catalyzed by Fe^{2+} in fentons reaction[53]. SSE and SCE possess higher ability to chelate Fe^{2+}, with respect to LSE and LCE. Abundance of Fe^{2+} ions in the *striatum* nervous structure enhance the formation of oxidative species in the brain through the Fenton reaction[54].Kusano and Ferrari also suggested that substances exhibitingmetal chelating activitiesmay be used to delay the rate of progression and onset of AD[55]. Thus,the seed may be of more importance than the leaf in the preventing AD.

Total phenol Content

Similarly with the above trend, the total phenol content values are higher in the seed extracts (SSE and SCE; 238.943 ± 9.938905 and 175.8991 ± 2.289846 respectively) compared to the leaf extracts (LSE and LCE; 44.06248 ± 3.751449 and 31.10293 ± 0.876962 respectively), with SSE exhibiting the highest value and LCE the lowest values. Ranju *et al.*,also demonstrated that ethanol extract of A. precatorius seeds contained the higher level of total phenol[56]. Phenolic compounds are potential antioxidants and free radical scavengers, hence, total phenolic content in medicinal plantshave been shown to correlate with their antioxidant activity[57]. Significantly high amountof phenolic compounds found in soxhlet extract of seeds of *A. precatorius* as compared to the cold extraction may be due to the fact that the seeds are coated and less permeable to solvents leading to poor extraction of phenolic compounds in the cold extraction than the soxhlet extraction. This could account for the high antioxidant activity with respect to electron donating(TAC and FRAP) and metal chelating activity observed in this study for SSE compared to SCE. The higher antioxidant activity possessed by the seed as compared to the leaf gives it a higher edge in the prevention of oxidative stress which is implicated in the pathogenesis of Alzheimer's disease.

Total flavonoid content.

Leaf extracts (LSE and LCE) exhibited higher flavonoid content (206.9823 ± 14.08638 and 157.2195 ± 13.3002respectively) compared to the seed extracts SSE and SCE with flavonoid content of 139.336 ± 16.13793 and 81.79768 ± 14.58653 respectively; LSE exhibiting the highest value and SCE exhibiting the lowest value. This study is in line with that of Jain *et al.,* who demonstrated that the leaves of *A. precatorius* had the higher flavonoid content than the seeds[44]. Flavonoids are a type of phenolic compounds that show antioxidant activity and has considerable effects on human health[58]. They act as ROS by donating electrons, scavenging free radicals or chelating metals[59,60]. Although the leaves of contained low phenolic content, the high flavonoid content which may be the major phenol in the leaves may account for the antioxidant activities observed in the leaves of *A. precatorius*

Anticholinesterase activity

Higher anticholinesterase activity was observed in the seed extracts (SSE and SCE); with IC_{50} values of 2.823005 ± 0.899431 and 5.392917 ± 0.3383respectivelycompared to the leaf extracts(LSE and LCE) with IC_{50} values of7. 3929 ± 0.3793 and 9.052707 ± 1.600707respectively. SSE exhibiting the highest value and LCE exhibiting the lowest value.

Acetylcholine (ACh) is neurotransmitter liberated at nerve endings.Neurotransmitter disturbances and reduced cholinergic functions such as AD are identified among the pathological features in central nervous system disorders linked to prolonged deficiency of acetylcholine (ACh)[61,17].Defect in cholinergic function in AD can be reduced through the stimulation of cholinergic receptors or increasing cholinergic transmission in the central nervous system by the inhibition ofACh hydrolysis by means of AChE inhibitors[17].Saeedi *et al.,* made it known that studies on plant-derived compounds such as physostigmine, galantamine and huperzine indicated that AChE inhibitors could be demanded from natural extracts[62].

Thus *A. precatorius* seeds and leaves through their inhibition of AChE activity may provide an approach to the prevention and treatment of AD. The highest antioxidant activity exhibited by SSE may be responsible for its high anticholinesterase activity. In addition, antioxidant therapy has also been proven to be successful in improving cognitive function and behavioral deficits in patients with AD[63]. The highest anticholinesterase and antioxidant activities exhibited by SSE is an indication that the seed of *A. precatorius* may be more efficient in the treatment of AD than the leaves. Furthermore, Soxhelet extraction and thus application of heat may also enhance the activity of the seeds and leaves.Gotecha *et al*., have recently revealed in their anti-inflammatory studies of *Abrusprecatorius* seeds that the methanolic extract of *Shodhit* (detoxified) seeds, involving heating process significantly enhanced anti-inflammatory activity compared to *Ashodhit* (non-detoxified) seeds that does not involve the application of heat[64].

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