Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Chemical Profiling, Free Radical Scavenging and Anti-acetylcholinesterase Activities of Essential Oil from *Curcuma caesia* of Arunachal Pradesh, India

Sudarshana Borah, Priyanka Sarkar[1], Hemanta Kumar Sharma

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, [1]DBT Wellcome Lab, Asian Healthcare Foundation, Asian Institute of Gastroenterology, Hyderabad, Telangana, India

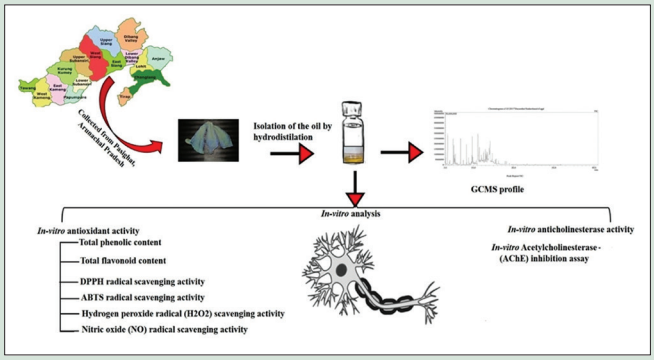
ABSTRACT

Background and Aim: The rhizomes of *Curcuma caesia* have traditional applications for pain healing and are an imperiled species of India. A study on investigation of *in vitro* anti-oxidant and acetylcholinesterase (AChE) inhibitory efficacy of essential oil from the rhizome part was carried out through extraction by hydrodistillation. **Materials and Methods:** The phyto components present in this volatile oil were identified by gas chromatography-mass spectrometric analysis and based on Spearman's correlation, the cooccurrence network of the 21 compounds were analyzed. *In vitro* anti-oxidant, anti-AChE activities were assessed with different concentrations of essential oil by spectrophotometric analysis, following the standard protocols. **Results:** The studies revealed that major compounds identified were Androsta-1,4-dien-3-one,17-(acetyloxy)-, (17.beta.)-Santanol acetate (16.11%), Eucalyptol (12.98%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) (8.96%), methyl 7,12-octadecadienoate (6.75%), and (+)-2-Bornanone (6.60%). The major interactions of terpenoidal compounds with other compounds give a picture that terpenoids have a very significant role as anti-oxidants and cholinesterase inhibitors. The essential oil showed the total phenolic content of 26.72 ± 0.38 mg/g of gallic acid equivalent and total flavonoid content of 18.92 ± 0.27 mg/g of quercetin equivalent (QE). 2, 2-diphenyl-1-picrylhydrazyl and 2, 2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid radical scavenging 50% inhibitory concentration (IC_{50}) values of the oil were 186.33 ± 0.46 µg/mL and 109.41 ± 0.43 µg/mL. The hydrogen peroxide and nitric oxide radical scavenging IC_{50s} were 103.45 ± 0.58 µg/mL and 190.55 ± 0.75 µg/mL, respectively. The AChE inhibitory IC_{50} value was 156.33 ± 0.44 µg/mL. **Conclusion:** The study demonstrated on essential oil extracted from rhizomes of *C. caesia* indigenous to Arunachal Pradesh, India, has moderate anti-oxidant property and would be a valuable non-toxic source for managing neurodegenerative diseases.

Key words: Cholinergic, ethnomedicinal, hydrodistillation, spectrophotometric, terpenoid

SUMMARY

- Along with the traditional applications for pain healing, *Curcuma caesia* has been scientifically evidenced to have anti-oxidant, smooth muscle relaxant, anxiolytic, and antidepressant properties which may contribute to its protection against neurodegenerative disorders. To have a detailed knowledge on this property, a study has been conducted exploring on the phytoconstituents of the essential oil from Pasighat, Arunachal Pradesh of Northeast region for its anti-oxidant and anti-acetylcholinesterase (AChE) activities for the treatment of Alzheimer's disease that would be a novel approach. Pasighat district in Arunachal Pradesh comprises archetypally low land and humid subtropical climate which are exclusively channel surrounded by high hills resting on three sides that forms Pasighat as idyllic to catch the attention of rain and making the land more fertile. Essential oil extraction by hydrodistillation from the rhizomes was identified by gas chromatography-mass spectrometric analysis. *In vitro* anti-oxidant, anti-AChE activities were assessed with its different concentrations by spectrophotometric analysis. The major compounds identified were Androsta-1,4-dien-3-one,17-(acetyloxy)-,(17.beta.)-Santanol acetate (16.11%), Eucalyptol (12.98%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl, (1a.alpha., 3a.beta., 6a.beta., 6b.alpha) (8.96%), Methyl 7,12-octadecadienoate (6.75%), and (+)-2-Bornanone (6.60%). The essential oil showed total phenolic content of 26.72 ± 0.38 mg/g of gallic acid equivalent and total flavonoid content of 18.92 ± 0.27 mg/g of QE. 2, 2-diphenyl-1-picrylhydrazyl and 2, 2′-azino-bis- (3-ethylbenzothiazoline-6-sulphonic acid radical scavenging 50% inhibitory concentration (IC_{50}) values of the oil were 186.33 ± 0.46 µg/mL and 109.41 ± 0.43 µg/mL. The hydrogen peroxide and nitric oxide radical scavenging IC_{50s} were 103.45 ± 0.58 µg/mL and 190.55 ± 0.75 µg/mL. The AChE inhibitory IC_{50} value was 156.33 ± 0.44 µg/mL. Spearman's correlation of the major compounds was detected and found that terpenoids have the foremost correlation with other compounds and thus, terpenoid present in the essential oil of *C. caesia* rhizomes have a very significant role as free radical scavengers and AChE inhibitors.



Abbreviations Used: AChE: Acetylcholinesterase; AD: Alzheimer's disease; CNS: Central nervous system; CCEO: *Curcuma caesia* essential oil.

Correspondence:
Dr. Hemanta Kumar Sharma,
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh - 786 004, Assam, India.
E-mail: hemantasharma123@yahoo.co.in

DOI: 10.4103/pr.pr_84_19

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

The cholinergic role is associated with oxidative stress such as lipid peroxidation and protein oxidation occupies an important role in cellular homeostasis of Alzheimer's disease (AD).[1-3] Inhibition of cholinesterase enzyme is required for the treatment of AD. AD, a leading civic health concern universally, is characterized by the abnormal deposition of

Cite this article as: Borah S, Sarkar P, Sharma HK. Chemical profiling, free radical scavenging and anti-acetylcholinesterase activities of essential oil from *Curcuma caesia* of Arunachal Pradesh, India. Phcog Res 2020;12:76-84.

Aβ peptide, and intracellular accumulation of neurofibrillary tangles of hyperphosphorylated τ proteins associated with dementia. The accumulation of these abnormal proteins transforms and inhibits the action of other useful proteins of our cholinergic system. The pathological attributes that occur in the central nervous system are due to oxidative, inflammatory and genetic factors, among these oxidative stress causes regular levels of pathogenic consequences of the disease. The cholinergic hypothesis suggests that deficiency of neurotransmitter acetylcholine is associated with the cognitive and behavioral symptoms of the affected patients. The enzyme acetylcholinesterase (AChE) plays an important role in activities associated with the central and peripheral nervous systems since it effectuates the hydrolysis of the neurotransmitter acetylcholine, thus producing choline and acetate.[4] The cholinergic hypothesis which has turned out to be a standard loom in the symptomatic treatment of AD depicts that inhibition of both AChE is necessitated to conserve the levels of acetylcholine and alleviate the cholinergic function. At present, numerous plants with anti-oxidant property have been recognized as containing AChE inhibitory activity.[5-7]

Ever since the primitive epoch, the medicinal values of plants have been explored in the modern scientific advancements worldwide, on account of their potent anti-oxidant activities. Anti-oxidants countervail the actions of free radicals responsible for oxidative damage, which is by interfering with the oxidation process and also by behaving as oxygen scavengers.[8,9] Free radicals have an enhanced substantiation in the etiology of aging and neurodegenerative diseases.[10] These are the compounds with one or more unpaired electrons in its outermost shells.[11] The role of these unpaired electrons is to make such species unbalanced and reactive with other molecules because of the presence of unpaired electrons to make them pair up with their electrons and thereby produce additionally stable compounds.[12] These molecules are highly reactive and able to exist in a self-regulating manner.[13] Reactive oxygen species (ROS) are constantly produced inside our body as the most hazardous free radicals. Anti-oxidants inside our body play the most important role of detoxifying these toxic ROS, even though the excess accumulation of ROS can cause the unequal amount of anti-oxidant defense which without any doubt may influence and carry oxidative damage to lipids, proteins, lipoproteins, and DNA.[14] ROS formed inside our physiological system, for instance, hydroxyl radical, superoxide anion and hydrogen peroxide, are extremely reactive and possibly damage transient chemical species. Consequences of damage in tissues that results due to an imbalance amid ROS-generating and scavenging systems that have been impeached in the pathological process of a range of diseases such as hypertension, atherosclerosis, diabetes mellitus, aging, AD, cancer, and AIDS.[15-17] Oxidative stress increases in the brain initiating with aging processes that is activated by an imbalance in the redox condition relating to the excess generation of ROS that causes disturbance of the brain's anti-oxidant systems.[18,19] In patients suffering from AD, free radicals may possibly generate from the β-amyloid peptides and advanced glycation end products. Scientific reports have suggested that AD progression can be prohibited by the interference of natural anti-oxidants from a variety of plant sources from different geographical locations that hold out resistance against oxidative stress by scavenging the free radicals and by other mechanisms.[20,21] In recent epoch, investigation has been implemented in the exploration of natural antioxidants from herbs. Polyphenols are natural anti-oxidants that inhibit the proliferation of free radical response to defend our body from ailment and impede lipid oxidative rancidity.[22-24] Consequently, various investigations are being carried on these ethnomedicinal plant resources for effective natural anti-oxidants to reinstate the synthetic drugs.[25]

Curcuma caesia Roxb., (Family: Zingiberaceae) is a perennial aromatic herb with bluish-black colored rhizome that has a bitter taste with pungent smell, commonly known as *"Black Turmeric"* and locally known as "*Kolaa Haladhee*" (in Assamese and Adi language). This ethnomedicinal plant is indigenous to northeast India.[26,27] It also originates in some parts of the hilly regions of east and the west Godavari, some districts of Andhra Pradesh, central parts of India and in other Asian countries. Conventionally, the plant rhizome is effective as a pain reliever and therefore useful in treating rheumatoid arthritis pains, and headache. It also has some other beneficial as a high anti-oxidant, carminative, antiemetic, anti-diarrheal, etc. In addition, the rhizomes are also employed in the treatment of skin disorders such as leukoderma, as a diuretic, in the treatment of piles, bronchitis, asthma, etc.[28-30] In Manipur regions of Northeastern India, the rhizome paste is conventionally used in the treatment of bruises and for relief of rheumatic pains.[31] The decoction of fresh rhizome is used in the treatment of diarrhea by Adi tribes of Arunachal Pradesh. On the other hand, the paste of fresh rhizome is used by Khamti tribes of Lohit district in the treatment of snakebite and scorpion sting.[32-34] This plant is widely used in Ayurvedic, Unani, and Siddha System of Medicine as a traditional pain healer. The essential oil of this plant possesses antimicrobial activity.[26,34] Besides, this plant also has a major amount of useful phytoconstituents such as terpenoids and polyphenols that are absent in *Curcuma longa*. Scientific reports have evidenced that this plant has high anti-oxidant, smooth muscle relaxant, anxiolytic, and antidepressant-like activities which may contribute to its protection against neurodegenerative disorders; therefore, it is thought to be worthy to explore the phytoconstituents of the essential oil from northeast region and investigate on the anti-oxidant and anti-AChE activities of the plant rhizome as a novel approach for the treatment of AD.[35]

MATERIALS AND METHODS

Plant collection and preparation

The rhizomes of *C. caesia* Roxb. were collected from Pasighat district of Arunachal Pradesh, India (Latitude: 27° 32/N to 29° 20/N and Longitude: 93° 48/E to 95° 36/E, Elevation: 502 ft above mean sea level). The plant specimen was authenticated by the Botanical Survey of India, Eastern Regional Centre, Shillong, of specimen no. DU/HS/SB/2017/01 and Reference No. BSI/ERC/Tech/2017/114. A herbarium of the plant specimen has been deposited in the herbarium repository of the Department of Pharmaceutical Sciences, Dibrugarh University.

Extraction of essential oil

The essential oil was extracted from the freshly cut rhizomes of *C. caesia* essential oil (CCEO) by hydrodistillation for 8 h using a Clevenger type apparatus. A pale yellow colored oil was collected in sterilized amber colored glass vial and stored at 4°C for further analysis.[36,37]

Yield of essential oil *Curcuma caesia* essential oil

The yield of CCEO was calculated by using the following equation:

$$\text{Percentage yield of CCEO} = \frac{\text{Weight of extracted CCEO}}{\text{Weight of crude rhizomes}} \times 100\% \quad \text{(Equation 1)}$$

Gas chromatography and mass spectrometry

Constituents of CCEO were quantified and identified by gas chromatography-mass spectrometry (GC-MS) on an Agilent 5921A gas chromatograph hooked to an Agilent 5975 mass selective detector. GC was outfitted with HP-5MS (30 m × 250 μm × 0.25 μm). Initially, the oven temperature was held at 50°C for 1 min and ramped at 20°C/min to 200°C for 1 min. The injector temperature was maintained at 280°C. The sample of 10 μL volume was diluted to 1% with methanol and then

injected by the splitless method. Helium was chosen as a carrier gas at the flow rate of 1 mL/min. Scanning of spectra was done in the range from 50 m/z to 550 m/z at 3 scans per second. The chromatogram obtained was compared with the data stored up in NIST08 (NIST, Gaithersburg, MD, USA) and W8N08 (John Wiley and Sons, Inc., USA). Relative percentages were determined for each constituent of the essential oil from the GCMS peak area % information.

Compound network plot analysis

The cooccurrence network of the compounds was obtained, based on the Spearman's correlation of the GCMS profiles of the samples. The analysis was carried out in Statistical Package of Social Scientists (SPSS) Software (IBM SPSS 20, SPSS Inc., Chicago, IL) followed by the construction of the network using Cytoscape 3.5.0. The compounds, showing significant associations ($P \leq 0.01$) were taken for the network plot constructions. Prefuce force-directed layout was considered for the visualization of the networks.[38]

Determination of *in vitro* anti-oxidant potential

Total phenolic content

The total phenolic content of CCEO was analyzed using standard gallic acid (Sigma Aldrich, Chemical Co., Mumbai, India) at (1 mg/ml) and test sample solutions (1 mg/ml) of CCEO prepared at concentrations of 20, 40, 60, 80, and100 µg/ml. A volume of 1 ml of standard and each concentration of test solution was mixed through 5 ml of the folin–ciocalteu reagent (Loba Chemie, Pvt. Ltd., Mumbai, India) and 4 ml of sodium carbonate (Himedia Laboratories, Mumbai, India). Absorbance readings were calculated spectrophotometrically in triplicates at 765 nm. A volume of 1 ml of distilled water was mixed with 5 ml of the folin–ciocalteu reagent and 4 ml of sodium carbonate were taken as control. All measurements were performed in triplicates. The total phenolic content in the CCEO was expressed as gallic acid equivalents (GAEs) that was calculated by using the following formula.[39]

$$T = \frac{C \times V}{M} \qquad \text{(Equation 2)}$$

Where, T = Total phenolic contents, mg/g of CCEO in GAE, C = Concentration (mg/mL) of Gallic acid obtained from calibration curve, V = Volume of CCEO (mL), M = Weight (g) of CCEO.

Total flavonoid content

The total flavonoid content of CCEO was analyzed by means of aluminum nitrate (Sigma Aldrich, Chemical Co., Mumbai, India). Quercetin (Sigma Aldrich, Chemical Co., Mumbai, India) was used as the standard. A total of 1 mg of CCEO was added to 1 mL of 80% ethanol. An aliquot part containing 0.5 mL was added to each test tube containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate (Loba Chemie, Pvt. Ltd., Mumbai, India) and 4.3 mL of 80% ethanol. The supernatant was collected, and the absorbance values were measured at 415 nm after incubation at room temperature for 40 min. The total flavonoid content of CCEO was measured as µg quercetin equivalent (QE) by using the standard quercetin graph and by means of the given formula.[40]

$$T = \frac{C \times V}{M} \qquad \text{(Equation 3)}$$

Where, T = Total flavonoid content, mg/g CCEO, in Quercetin Equivalent (QE), C = Concentration (mg/mL) of Quercetin obtained from calibration curve, V = Volume of CCEO (mL), M = Weight (mg) of CCEO.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Ascorbic acid (Himedia Laboratories, Mumbai, India) was taken as a standard (1 mg/ml) and essential oil as test solutions (1 mg/ml). Test solution (CCEO) was prepared at concentrations of 20, 40, 60, 80, and 100 µg/ml. For the preparation of standard and test solutions, 1 ml of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich Chemical Co., Mumbai, India) solution was added to 2 ml of each diverse concentrations of standard and test solutions, vigorously shaken and was incubated in dark condition for 30 min at room temperature. Control was prepared by adding 1 ml of 0.2 mM DPPH solution to 2 ml of methanol and this solution was allowed to incubate at dark for 30 min at room temperature. Immediately, subsequent to 30 min, absorbance was measured at 517 nm taking methanol as blank using ultraviolet (UV)-visible spectrophotometer. The inhibitory percentage values were calculated by comparing the absorbance values of the control and test samples. All the tests were executed in triplicate. The potential to scavenge the DPPH radical was calculated as the inhibition percentage of free radical by the sample and standard by using the following formula:

$$\%\ \text{Inhibition of DPPH radicals} = Ao - At / Ao \times 100 \qquad \text{(Equation 4)}$$

Where A_O is the absorbance of the control and A_t is the absorbance of test and standard. The DPPH radical scavenging activity of the essential oil was evinced as 50% inhibitory concentration (IC_{50}). The IC_{50} value is specified as the concentration of CCEO (µg/mL) that inhibits by 50% the formation of DPPH free radicals.[17,41]

ABTS (2,2'- Azino-bis (3- ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity

Ascorbic acid was taken as a standard (1 mg/ml) and essential oil as test solutions (1 mg/ml). Both standard and test solution (CCEO) was prepared at concentrations of 50, 100, 150, 200, 250, and 500 µg/ml. The ABTS (Sigma-Aldrich Chemical Co., Mumbai, India) solution was dissolved in aqueous solution to get 7 mM concentration. The production of ABTS radical cation ($ABTS^{*+}$) was made following reaction between ABTS stock solution with 2.45 mM potassium pursulfate (final concentration). The mixture was allowed to stand in the dark at 28 ± 2°C for 16 h before utilization. The test samples were analyzed, by diluting the $ABTS^{*+}$ solution into absolute methanol to an absorbance of 0.700 ± 0.02 at 734 nm and equilibrized at 30°C. A_0 was notified as reagent blank and its reading was recorded. Immediate subsequent to the addition of 2.0 mL of diluted $ABTS^{*+}$ solution (A734 nm = 0.700 ± 0.02) to 20 µL of the test sample, the absorbance was noted at 30°C exactly for 6 min after initial mixing (At). Suitable solvent blanks were run in each one assay. All the tests were performed three times. The decrease in absorbance between A_0 and A_t were calculated as the percentage inhibition of ABTS free radicals by the essential oil according to the following formula:

$$\%\ \text{Inhibition of ABTS radicals} = Ao - At / Ao \times 100 \qquad \text{(Equation 5)}$$

Where Ao control is the absorbance of the control reaction and At is the absorbance of test and standard solutions. The anti-oxidant activity of the CCEO was expressed as $IC_{50.}$ The IC_{50} value is defined as the concentration (µg/mL) of CCEO that inhibits the formation of ABTS radicals by 50%.[42]

Hydrogen peroxide radical (H_2O_2) scavenging activity

Ascorbic acid was chosen as a standard (1 mg/ml) and essential oil (CCEO) as test solutions (1 mg/ml) for this assay. Both standard ascorbic acid (1 mg/ml) and CCEO solutions (1 mg/ml) were prepared at concentrations of 50, 100, 200, 400, 600, 800, and 1000 µg/ml. To 1 ml of ascorbic acid and CCEO solution 0.6 ml of H_2O_2 (Himedia Laboratories,

Mumbai, India) was added. After 10 min, the absorbance value of H_2O_2 was determined at 230 nm against a blank solution containing the phosphate buffer without H_2O_2. To 1 ml of phosphate buffer (Loba Chemie, Pvt. Ltd., Mumbai, India) solution, 0.6 ml of H_2O_2 was added and this was used as control. The admixtures were carried out in triplicate spectrophotometrically. The percentage inhibition of H_2O_2 free radical scavenging activity of ascorbic acid and CCEO were calculated by means of the given formula,

$$\% \text{ Inhibition of } H_2O_2 \text{ radicals} = Ao - At / Ao \times 10 \quad \text{(Equation 6)}$$

Where A_0 is the absorbance of the control mixture and A_t is the absorbance of CCEO. The anti-oxidant activity of the CCEO is expressed as IC_{50} value. The IC_{50} value is defined as the concentration (μg/ml) of CCEO that inhibits the formation of H_2O_2 radicals by 50%.[17]

Nitric oxide radical scavenging activity

Ascorbic acid was chosen as a standard (1 mg/ml) and essential oil (CCEO) as test solutions (1 mg/ml) for this assay. Both standard ascorbic acid (1 mg/ml) and CCEO solutions (1 mg/ml) were prepared at concentrations of 80, 160, 320, 500, 800, and 1000 μg/ml. To 1 ml of standard and CCEO solutions 1 ml of sodium nitroprusside (Himedia Laboratories, Mumbai, India) and 1 ml of phosphate buffer (saline pH 7.4 were added and incubated at 25°C for 150 min. Subsequent to incubation, 1 ml of the above admixture was blended with 1 ml of Griess reagent. This admixture was allowed to stand in darkness for 30 min and afterward absorbance was measured at 546 nm. To 1 ml of distilled water, 1 ml of sodium nitroprusside solution, and 1 ml of phosphate buffer were mixed together and kept at incubation for 150 min at 25°C. Subsequent to incubation 1 ml of Griess reagent was added to it and then allowed to stand in darkness for 30 min and then absorbance was measured at 546 nm, which was taken as control. The absorbance was measured spectrophotometrically in triplicates and then free radical scavenging activity was expressed as the percentage inhibition of free radical by ascorbic acid and CCEO and was calculated by the given formula:

$$\% \text{ Inhibition of NO radicals} = Ao - At / Ao \times 100 \quad \text{(Equation 7)}$$

Where, A_O is the absorbance of the control and At is the absorbance of ascorbic acid and CCEO. Nitric oxide (NO) free radical scavenging activity of CCEO was expressed as IC_{50} value. The IC_{50} value can be defined as the concentration (μg/mL) of CCEO that inhibits the formation of NO by 50%.[17]

In vitro acetylcholinesterase inhibition assay

The AChE inhibition assay was determined using the spectrophotometric method by following Ellman's standard protocol with trivial modifications in it. In this assay in a total volume of 100 μL, 60 μL of Tris-HCl buffer 0.1 M concentration and pH 8 was added to a 10 μL of solution of essential oil in Tween 80 (0.5% v/v), with different concentrations and 25 μL of enzyme (Electric eel AChE, Sigma-Aldrich Chemical Co., Mumbai, India) solution. The solutions were incubated for 15 min at room temperature. Immediate after incubation, 10 μL of a solution of acetylthiocholine iodide (Sigma-Aldrich Chemical Co., Mumbai, India) of 0.5 mM concentration and 10 μL of 5,5-dithiobis-2-nitrobenzoicacid (DTNB) (Sigma-Aldrich Chemical Co., Mumbai, India) of 3 mM concentration were added, and the final mixture was incubated for 30 min, at room temperature. Absorbance of the mixture was measured at 412 nm in a UV-visible spectrophotometer (Shimadzu, Japan). Rivastigmine (Sigma-Aldrich Chemical Co., Mumbai, India) of 0.5 mM concentration was added at each well and used as a positive control. All experiments were performed in triplicate with their respective positive control. The percentage inhibition (I) of AChE was calculated with the help of Equation 8.[43,44]

$$I = (C - T) / C \times 100 \quad \text{(Equation 8)}$$

Where, C is the activity of enzyme without test sample and T is the activity of the enzyme with test sample. The concentration of the test sample that inhibited the hydrolysis of acetylthiocholine (substrate) by 50%, i.e., IC_{50} was determined by monitoring the effect of increasing concentrations of the sample.

RESULTS

The fresh rhizome pieces of 3 kg were hydrodistilled for oil isolation and produced a viscous, pale yellow volatile oil weighing about 5 mL. The percentage yield of oil was 60%. Based on GCMS analysis, the composition of essential oil isolated from the rhizomes of *C. caesia* Roxb. was significantly different and constituted of Androsta-1,4-dien-3-one, 17-(acetyloxy)-, (17.beta.)-Santanol acetate (16.11%), Eucalyptol (12.98%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl,(1a. alpha.,3a. beta.,6a. beta.,6b. alpha) (8.96%), Methyl 7,12-octadecadienoate (6.75%), (+)-2-Bornanone (6.60%), L-Bornyl Acetate (4.47%), Isogermafurene (4.34%), Isoborneol (3.5%), and Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester (3.28%) as listed in Table 1. The major constituents present in essential oil of this plant was dependent on its geographical distribution. In Figure 1, the gas chromatography-mass spectrometric chromatogram of CCEO has been demonstrated. Further, major interactions among terpenoids were observed in the correlation analysis as shown in Figure 2. Isoborneol, (+)-2-Bornanone, alpha-Terpineol, and eucalyptol were observed to have positive interactions with each other and common negative interactions with acetic acid and Methyl 7,12-octadecadienoate. Interestingly, selina-6-en-4-ol has negative correlation with isobroneol, (+)-2-borneone, alpha-terpeneol, eucalyptol but positive correlation with acetic acid and methyl 7, 12-octadecandienoate. In addition, the other compounds forming the major networks were observed as having positive associations with either acetic acid or terpenoid compounds. From the *in vitro* enzyme inhibitory assays, percentage inhibition and IC_{50} were calculated. The total phenolic content of CCEO was measured to be 26.72 ± 0.38 mg/g of GAE as depicted in Figure 3 from the calibration curve of gallic acid. The total flavonoid content of CCEO was calculated to be 18.92 ± 0.27 mg/g of QAE as shown in Figure 4 from the calibration curve of quercetin. The DPPH radical scavenging IC_{50} value of CCEO was 186.33 ± 0.46 μg/mL against ascorbic acid of 9.33 ± 0.65 μg/mL.

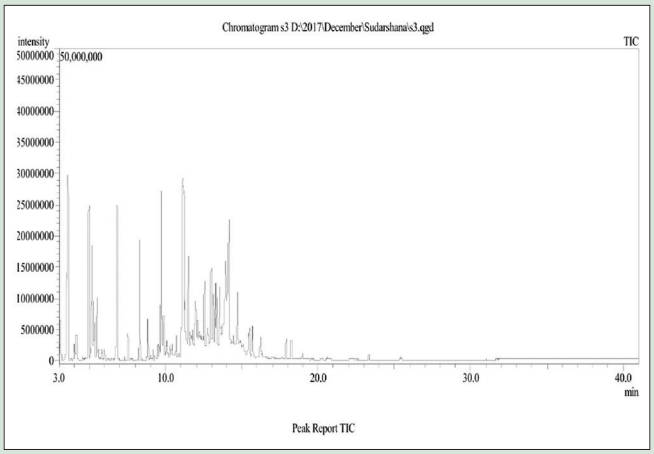


Figure 1: Total ion chromatogram for CCEO using gas chromatography-mass spectrometry

Table 1: List of major constituents of the essential oil obtained from the rhizomes of *Curcuma caesia* indigenous to Arunachal Pradesh, India

Name of the compound	Relative content (%)	Retention time (min)
Androsta-1,4-dien-3-one, 17-(acetyloxy)-, (17.beta.)- Santanol acetate	16.11	11.116
Eucalyptol	12.98	3.589
Cycloprop[e] indene-1a, 2(1H)-dicarboxaldehyde, 3a, 4,5,6,6a, 6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha)	8.96	14.19
Methyl 7,12-octadecadienoate	6.75	13.94
(+)-2-Bornanone	6.60	4.965
L-Bornyl Acetate or Bicyclo[2.2.1] heptan-2-ol, 1,7,7-trimethyl-, acetate	4.47	5.28
Isogermafurene or Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	4.34	9.74
Isoborneol	3.5	5.18
Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester	3.28	11.49
(-)- beta- Elemene or Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-, [1S-(1. alpha.,2.beta.,4.beta.)]-	2.59	8.30
Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	1.91	13.55
Davana ether	1.84	13.28
3,5-Dimethyl-1,4-diallylpyrazole	1.82	14.72
(E, E)- Germacrone or 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E, E)-	1.74	12.55
Selina-6-en-4-ol	1.69	11.99
alpha.-Terpineol	1.59	5.51
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-7.alfa.,21.beta.-28,30-Bisnorhopane	1.55	6.83
2,4a-Methanonaphthalen-7(4aH)-one, 1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-, (2s-cis)-	1.34	18.25
(-)- β- Pinene	1.18	3.09
5,8-Dihydroxy-4a-methyl-4,4a, 4b, 5,6,7,8,8a, 9,10-decahydro-2(3H)-phenanthrenone	1.06	15.68

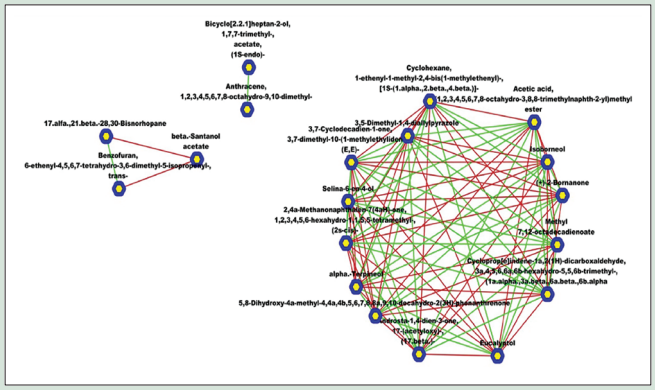


Figure 2: Compound-Compound network correlation

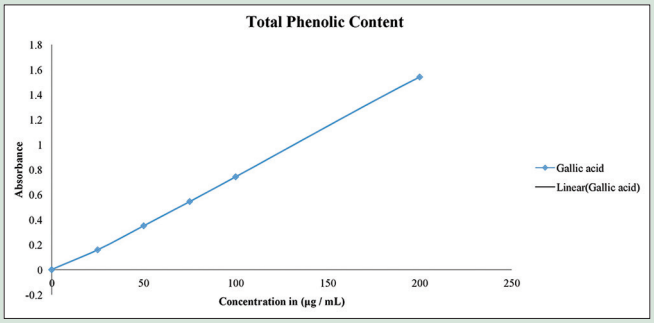


Figure 3: Standard calibration curve of gallic acid. The investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007. The standard calibration curve was plotted and the equation of gallic acid was established as: y = 0.007x -0.026 with R^2 = 0.999

The scavenging activity of ABTS by essential oil was found to be higher 109.41 ± 0.43 µg/mL than that of DPPH against the standard ascorbic acid value of 11.17 ± 0.46 µg/mL. Figures 5 and 6 illustrate the % DPPH and ABTS radicals scavenging activities of essential oil (CCEO) against standard ascorbic acid. The H_2O_2 radical scavenging IC_{50} value of CCEO was 103.45 ± 0.58 µg/mL against ascorbic acid of 74.97 ± 1.6 µg/mL as illustrated in Figure 7. The NO radical scavenging IC_{50} value of CCEO was 190.55 ± 0.75 µg/mL against ascorbic acid of 11 ± 0.06 µg/mL as illustrated in Figure 8. The CCEO showed AChE inhibition with an IC_{50} value of 156.33 ± 0.44 µg/mL against rivastigmine that showed AChE inhibition with an IC_{50} value of 2.91 ± 0.18 µg/mL as depicted in Table 2.

DISCUSSION

Northeast India is a hub of medicinal plants. Various plants are found with diverse medicinal properties in this region. Arunachal Pradesh, a state of richness in flora and fauna is situated in the Northeastern margin of India. Pasighat district in Arunachal Pradesh comprises archetypally low land and humid subtropical climate, which are exclusively channel surrounded by high hills resting on three sides that forms Pasighat as idyllic to catch the attention of rain-bearing clouds from the Assam plains. In the land of Pasighat, there is heavy rainfall from June to September. During winters, a tough chilly dehydrated wind from the northeastern regions of the Siberian High makes Pasighat fog-free. During cold seasons usually from November to February, the climate is warm and clear, at the same time as from March to May, an escalating frequency of heavy thundershowers with very warm to hot, humid climate.[15] The composition of CCEO was found to be significantly different from the earlier reported scientific data by different researchers because of the disparity in maturity of the rhizomes and in geographical locations. Initially, in 1940, Dutt identified the essential oil to contain 76.6% δ-camphor but, Bannerjee *et al.*, in 1984 had reported on the presence of (+)-linalool (20.42%), ocimene (15.66%), 1-ar-curcumene (14.84%), zingiberol (12.60%), 1,8-cineole (9.06%), α-borneol (7%), and d-camphor (18.88%) as major constituents, except Rastogi *et al.*, who have found the absence of d-camphor., in the rhizomes. Behura, in 2000, had identified the constituents to be α-pinene (0.40%), β-ocimene (2.1%), camphor (7.73%), linalool (0.99%), caryophyllene (3.15%), borneol (4.3%), camphene (1.67%), anethole (1.79%), and cis-b-ocimene (14.54%). Pandey and *et al.*, in 2003, reported on the presence of camphor (28.3%), ar-tumerone (12.3%), (Z)-Ocimene (8.2%), 1-ar-curcumene (6.8%),

1,8-cineole (5.3%), elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%), and curcumene (2.82%). Paliwal, in 2011, had reported on 1,8-cineole (27.48%), camphor (14-28.3%), and ar-tumerone (12.3%). Besides, work carried out on the essential oil of its leaves by Behura and Srivastava in 2004 and Borah *et al.* in 2018 provides a strong base to further explore and repurpose the essential oil from its rhizomes based on geographical collection. The absence of camphor in our report gives us an apparent depiction that the rhizomes grown in Arunachal Pradesh of northeast region of India is deficient of camphor; however, the presence of camphor derivatives have been found in our CCEO sample[45-52] The rhizome being the most effective part of this plant has been chosen for further repurposing on its anti-oxidant and AChE inhibitory activities. Scientific evidence have suggested that terpenoids from natural source have a significance as neuroprotective. Work done by Mu *et al.*, in 2007 have reported on the neuroprotective effects of terpenoids and other phytoconstituents, namely flavonoids extracted from *Ginkgo biloba* that also produces synergistic effect in mixture. Kanhere *et al.*, in 2013, have established that terpenoid fraction from natural source *Hygrophila auriculata* has neuroprotective and anti-oxidant potential. In another literature, Karolina *et al.*, in 2017, have found that volatile terpenes, namely eucalyptol, citral, linalool, ocimene,α-pinene, etc., have AChE inhibitory activity and hence can be used as potential drug leads in AD.[7,53,54] In our report, mono- and di-terpenes forming the major networks are the potential anti-oxidants in the essential oil that would interrelate with other anti-oxidants of our physiological system. The redox properties of phenolics enable it to behave as an anti-oxidant which essentially plays a significant role in adsorption and neutralization of free radicals, decomposing peroxides or quenching of singlet and triplet oxygen.[55] The assessment of total phenolic content in the essential oil of *C. caesia* was done by using the Folin–Ciocalteu reagent. Phenolic compounds exhibit an imperative function as anti-inflammatory, anti-allergic, antibacterial, antiviral,

Table 2: Acetylcholinesterase inhibitory activities of *Curcuma caesia* essential oil in comparison with rivastigmine as standard drug

Plant extracts	AChE percentage inhibitory concentrations at 1 mg/mL (mean %±SEM)	AChE IC_{50} at (µg/mL±SEM)
Essential oil	74.27%±0.72	156.33±0.44
Rivastigmine	92.88%±0.92	2.91±0.18

AChE: Acetylcholinesterase; SEM: Standard error of the mean; IC_{50}: 50% inhibitory concentration

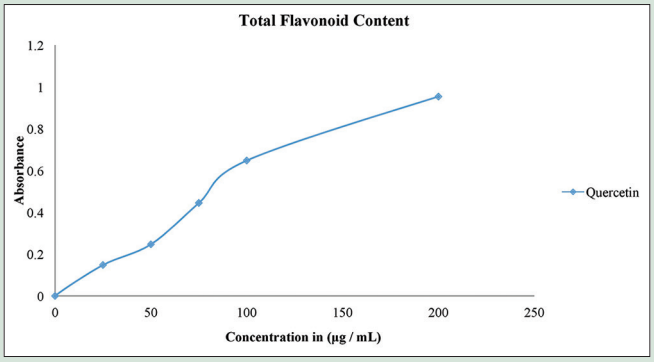


Figure 4: Standard calibration curve of quercetin. The investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007. The standard calibration curve was plotted and the equation of quercetin was found to be: y = 0.004x +0.041 with $R^2 = 0.96$

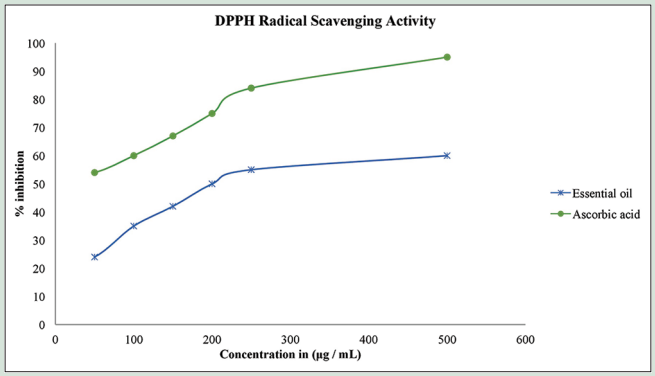


Figure 5: % DPPH scavenging activity of essential oil (CCEO) against standard ascorbic acid. Data are expressed as mean ± standard error of the mean the investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007 with $R^2 = 0.982$

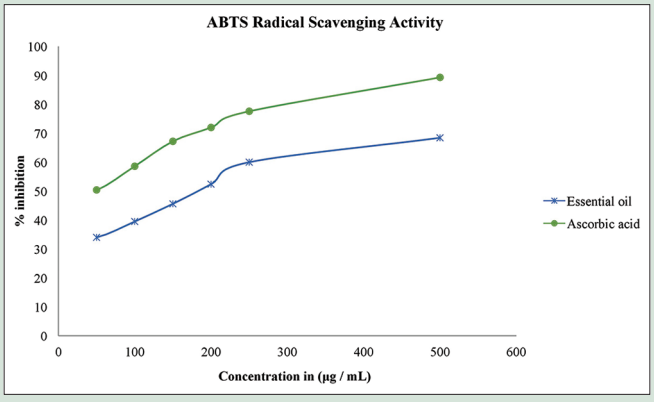


Figure 6: % ABTS scavenging activity of essential oil (CCEO) against standard ascorbic acid. Data are expressed as mean ± standard error of the mean the investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007 with $R^2 = 0.927$

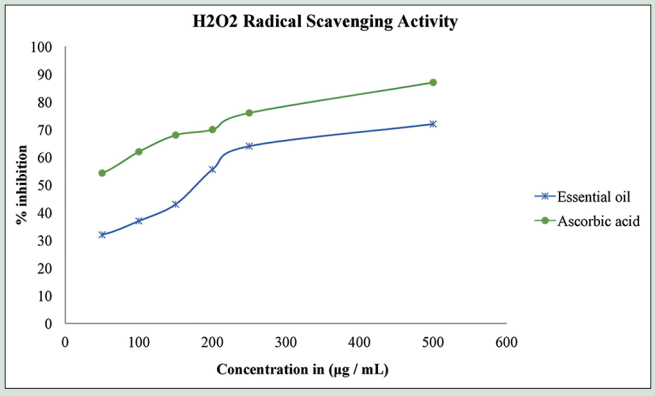


Figure 7: % Hydrogen peroxide radical scavenging activity of essential oil (CCEO) against standard ascorbic acid. Data are expressed as mean ± standard error of the mean the investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007 with $R^2 = 0.891$

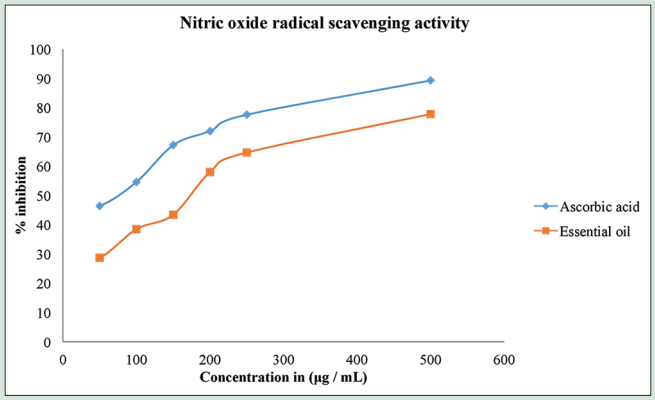


Figure 8: % Nitric oxide radical scavenging activity of essential oil (CCEO) against standard ascorbic acid. Data are expressed as mean ± standard error of the mean the investigational observations were carried out in triplicates for each enzymatic assay and hence, IC_{50} values were designed out with Microsoft Excel, 2007 with $R^2 = 0.832$

vasodilatory, and antithrombotic. More to the point, it also shows the signs of free radical scavenging properties by means of through chelation or scavenging process. Flavonoids are a huge class of benzopyrone derivatives, omnipresent in plants, and demonstrate anti-oxidant activity.[40,55] These compounds encompass the majority and most assorted group of polyphenols as plant secondary metabolites. Total flavonoid content could be expressed as µg of QEs/mg of extract. DPPH acts as a constant free-radical at room temperature and recognizes hydrogen radical or electron to develop into a constant diamagnetic molecule.[17] The potential of DPPH to reduce was measured by the reduction in its absorbance at 517 nm, which is persuaded by anti-oxidants. Iron has the ability to produce free radicals from peroxides by Fenton reactions, and reduction of the Fe^{2+} concentration in the Fenton reaction gives fortification next to oxidative harm. The addition of different concentrations of essential oil obstructs with the ferrous ferrozine complex and reduces the formation of the red-colored complexs with the escalating concentration. Proton radical scavenging is an important attribute of anti-oxidants. ABTS is a protonated radical that has features of absorbance maxima at 734 nm that reduces with scavenging of the proton radicals.[42,56] ABTS free radical cation could be fabricated in the constant form by means of potassium persulfate. Immediately subsequent to have obtained the constant absorbance value, the CCEO has been added to the reaction medium and the anti-oxidant power was measured by studying decolorization. Several factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals.[56] Similarly, Re *et al.*,[56] found that some compounds that have $ABTS^{\cdot+}$ scavenging activity could not scavenge DPPH. A correlation between these two models was observable in our study. This further showed the potential of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathologic damage. Hydrogen peroxide is reactive and can also sporadically be toxic to cell as it endows with hydroxyl radical in the cells. Consequently removing H_2O_2 and O^{2-} is very important for the protection of food systems.[17] NO acts a significant role in an assortment of inflammatory progression; however, its excessive accumulation causes different kinds of diseases. NO toxicity increases to the highest degree when it reacts with superoxide radicals, thus forming the most reactive peroxynitrite anion.[57,58] In this experiment, CCEO competes with oxygen to react with NO and therefore inhibits nitrite formation. Acetylcholine neurotransmitter which signals through metabotropic muscarinic and ionotropic nicotinic receptors induces synaptic plasticity and transmission and maintains coordination of neurons. This transmission is lapsed through AChE enzyme which hydrolyzes and breaks down acetylcholine neurotransmitter into acetate and choline before it arrives at the receptors. Cholinergic dysfunction is correlated with neurodegenerative diseases and hence, its inhibition would help in restoring the cholinergic functions. The enzyme AChE is a primary acetylcholine neurotransmitter inhibitor in the pathophysiology of AD. Low intensity of acetylcholine is one of the primary factors of AD. AD, which reports for approximately 60-70% of dementia cases in individuals over 60 years of age, is a foremost civic health concern that progressively impinges on our elderly population universally. Drugs like anticholinesterases protract the action of acetylcholine by inhibiting the enzyme AChE, thus enhancing the acetylcholine levels. These drugs act at the cholinergic nerve endings and helps in slowing down the degenerative processes. The standard drug chosen for this study is rivastigmine. This drug has easy permeability through the blood–brain barrier but when encapsulated as a hard capsule this drug carried side-effects such as nausea, vomiting, and diarrhea. The mechanism involved in this experiment states that AChE catalyzes the hydrolysis of acetylthiocholine into acetate and thiocholine. On the other hand, thiocholine highly reacts with DTNB to produce a yellow color that can be quantitatively measured using a spectrophotometer. Synthetic drugs such as cholinesterase enzyme inhibitors (galantamine and donepezil), anti-epileptic agents (felbamate), calcium channel blockers (nifedipine), anxiolytics and antidepressants (aprazolam, chlordiazepoxide, clonazepam, diazepam, flurazepam; fluoxetine and nootropic agents (piracetam) slow the succession of this disease, but lack to cure completely. Synthetic anti-Alzheimer's drugs such as rivastigmine, donepezil, tacrine, galanthamine, pyridostigmine, and neostigmine cause side-effects such as diarrhea, hepatotoxicity, nausea, vomiting, fatigueness, insomnia, muscles cramps, and loss of appetite.[59-62] That is why researchers are giving emphasis on naturally derived compounds and their derivatives because such compounds carry less or no side-effects at all.

CONCLUSION

Results illustrated on anti-oxidant and anti-AChE activities of the essential oil isolated from the rhizomes *C. caesia* indigenous to northeast India is the first report, which establishes the scientific fact that the essential oil of this plant has moderate anti-oxidant property and would be a valuable toxic free natural source for the management of neurodegenerative diseases. The major interactions of terpenoidal compounds with other compounds give a picture that terpenoids have a very significant role as anti-oxidants and cholinesterase inhibitors. Further analysis on *in vivo* models and the development of a formulation would facilitate to discern its mechanism and discover a novel, effective neuroprotective herbal drug.

Acknowledgements

The authors express their thanks to Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, for financial support. The authors are thankful to Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, for authenticating the plant. The authors are also thankful to Director, Institute of Advanced Study in Science and Technology, Guwahati, Assam, for extending laboratory facility.

Financial support and sponsorship

This research work received fund from Council of Scientific and Industrial Research (CSIR), HRDG, New Delhi, under CSIR Direct SRF scheme vide file no. 09/857 (0010)/2018-EMR-I.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. Front Aging Neurosci 2010;2:12.
2. Méndez-Armenta M, Nava-Ruíz C, Juárez-Rebollar D, Rodríguez-Martínez E, Gómez PY. Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. Oxid Med Cell Longev 2014;2014:293689.
3. Guerreiro R, Bras J. The age factor in Alzheimer's disease. Genome Med 2015;7:106.
4. Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, *et al.* Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol 2007;109:359-63.
5. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
7. Kanhere R, Anjana A, Anbu J, Sumithra M, Kanhere R, Anjana A. Neuroprotective and antioxidant potential of terpenoid fraction from *Hygrophila auriculata* against transient global cerebral ischemia in rats. Pharm Biol 2013;51:181-9.
8. Li X, Moses RE, Jin J, Cao W, Caulin C. Stresses, aging, and agerelated disorders. Oxid Med Cell Longev 2014;2014:1-2. Article ID 320564. Doi: http://dx.doi.org/10.1155/2014/320564.
9. Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN, et al. Free radical damage in ischemiareperfusion injury: An obstacle in acute ischemic stroke after revascularization therapy. Oxid Med Cell Longev 2018;2018:1-17. Article ID 3804979. Doi: https://doi.org/10.1155/2018/3804979.
10. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998;78:547-81.
11. Kumar H, Lim HW, More SV, Kim BW, Koppula S, Kim IS, *et al.* The role of free radicals in the aging brain and Parkinson's disease: Convergence and parallelism. Int J Mol Sci 2012;13:10478-504.
12. Markesbery WR. The role of oxidative stress in Alzheimer disease. Arch Neurol 1999;56:1449-52.
13. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89-96.
14. Farber JL. Mechanisms of cell injury by activated oxygen species. Environ Health Perspect 1994;102 Suppl 10:17-24.
15. Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease. Oxid Med Cell Longev 2013;2013:316523.
16. Sasikumar V, Kalaisezhiyen P. Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from *Kedrostis foetidissima* (Jacq) Cogn. Biochem Anal Biochem 2014;3:1-7.
17. Mahato K, Kakoti BB, Borah S, Kumar M. *In-vitro* antioxidative potential of methanolic aerial extracts from three ethnomedicinal plants of Assam: A Comparative Study. JAPS 2015;5:111-6.
18. Fonseca-Santos B, Gremião MP, Chorilli M. Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. Int J Nanomedicine 2015;10:4981-5003.
19. S Schneider L. A critical review of cholinesterase inhibitors as a treatment modality in Alzheimer's disease. Dialogues Clin Neurosci 2000;2:111-28.
20. Srivastava RC. Traditional knowledge of Adi tribe of Arunachal Pradesh on plants. Ind J Trad Know 2009;8:146-53.
21. Koynova R, Tenchov B. Natural Product formulations for the prevention and treatment of Alzheimer's disease: A patent review. Recent Pat Drug Deliv Formul 2018;12:23-39.
22. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 2006;141:312-22.
23. Mohammed RS, El Souda SS, Taie HA, Moharam ME, Shaker KH. Antioxidant antimicrobial activities of flavonoids glycoside from *Leucaena leucocephala* leaves. JAPS 2015;5:138-47.
24. Subasree S. Role of Vitamin C and Vitamin E in health and disease. J Pharm Sci Res 2014;6:52-5.
25. Nusaiba SA, Murugan K. *In vitro* analysis on bactericidal screening and antioxidant potentiality of leaf and root extracts of *Thottea* siliquosa (Lam.) ding hou. An ethnobotanical plant. Asian Pac J Trop Biomed 2013;3:859-65.
26. Das S, Zaman K. *Curcuma caesia* roxb and it's medicinal uses: A review. Int J Res Pharm Chem 2013;3:370-5.
27. Sahu B, Kenwat R, Chandrakar S. Medicinal value of *Curcuma cassia* roxb?: An overview. UK J Pharm Biosci 2016;4:69-74.
28. Sarangthem K, Haokip MJ. Bioactive components in *Curcuma caesia* roxb. Grown in Manipur. Bioscan 2010;5:113-5.
29. Kagyung R, Gajurel P, Rethy P, Singh B. Ethnomedicinal plants used for gastro – Intestinal diseases by adi tribes of Dehang Debang biosphere reserve in Arunachal Pradesh. Indian J Tradit Know 2017;9:496-501.
30. Tag H, Das AK, Loyi H. Anti-inflammatory plants used by the khamti tribe of Lohit district in Eastern Arunachal Pradesh. India. Nat Prod Rad 2007;6:334-40.
31. Angel GR, Vimala B, Nambisan B. Antioxidant and antimicrobial activity of essential oils from nine starchy *Curcuma* species. Int J Curr Pharm Res 2012;4:45-7.
32. Reenu J, Azeez S, Bhageerathy C. *In vitro* antioxidant potential in sequential extracts of *Curcuma caesia* roxb. Rhizomes. Indian J Pharm Sci 2015;77:41-8.
33. Devi HP, Mazumder PB, Devi LP. Antioxidant and antimutagenic activity of *Curcuma caesia* Roxb. rhizome extracts. Toxicol Rep 2015;2:423-8.
34. Arulmozhi DK, Sridhar N, Veeranjaneyulu A, Arora SK. Preliminary mechanistic studies on the smooth muscle relaxant effect of hydroalcoholic extract of *Curcuma caesia*. J Herb Pharmacother 2006;6:117-24.
35. Lawand RV, Gandhi SV. Comparison of *Curcuma caesia* roxb. with other commonly used *Curcuma* species by HPTLC. J Pharmacog Phytochem 2013;2:126-31.
36. Pandey A, Tripathi S. Concept of standardization extraction and pre phytochemical screening strategies for herbal drug. J Pharmacog Phytochem JPP 2014;2:115-9.
37. Mukunthan KS, Kumar NVA, Balaji S, Trupti NP. Analysis of essential oil constituents in rhizome of *Curcuma caesia* roxb. from South India. J Essent Oil Bear 2014;17:647-51.
38. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al.* Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498-504.
39. Silva S, Gomes L, Leitao F, Coelho AV, Boas LV. Phenolic compounds and antioxidant activity of *Olea europaea L.* Fruits and leaves. Food Sci Tech Int 2006;12:385-96.
40. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. J Biotech 2008;7:3188-92.
41. Shalaby EA, Shanab SMM. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. Indian J Mar Sci 2013;42:556-64.
42. haipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS DPPH FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006;19:669-75.
43. Ellman GL. A colorimetric method for determining low concentrations of mercaptans. Arch Biochem Biophys 1958;74:443-50.
44. Kostelnik A, Cegan A, Pohanka M. Acetylcholinesterase inhibitors assay using colorimetric ph sensitive strips and image analysis by a smartphone. Int J Anal Chem 2017;2017:3712384.
45. Dutt S. Indian Soap. J Chem Abs 1940;6:248-55.
46. Bannerjee AK, Kaul VK, Nigam SS. Essenze Deriv. Argum 1984;54:117-21.
47. Rastogi RP, Malhotra BN. Compendium of Indian Medicinal Plant CDRI. Vol. 199. Lucknow and Publication and Information Directorate, New Delhi; 1995. p. 241.
48. Behura S. Gas chromatographic evaluation of *Curcuma* essential oils. In: Ramana KV, Santosh JE, Nirmal Babu K, Krishnamurthy KS, Kumar A, editors. Spices and Aromatic Plants – Challenges and Opportunities in the New Century. Proceedings of the Centennial Conference on Spices and Aromatic Plants. Calicut, Kerala: Indian Institute of Spices Research; 2000. p. 291-2.
49. Pandey AK, Chowdhary AR. Volatile constituents of rhizome oil of *Curcuma caesia* from central India. Flavour Fragr J 2003;18:463-5.
50. Paliwal P, Pancholi SS, Patel RK. Pharmacognostic parameters for evaluation of the rhizomes of *Curcuma caesia*. J Adv Pharm Technol Res 2011;2:56-61.
51. Behura S, Srivastava VK. Essential oils of leaves of *Curcuma* species. J Essent Oil Res 2004;16:109-10.
52. Borah A, Paw M, Gogoi, R, Loying, R, Sarma N, Munda S, *et al.* Chemical composition, antioxidant, antiinflammatory, antimicrobial and *in vitro* cytotoxic efficacy of essential oil of *Curcuma caesia* Roxb. Leaves: An endangered medicinal plant of North East India. Ind Crops Prod 2018;129:44854. Doi: https://doi.org/10.1016/j.indcrop.2018.12.035.
53. Mu L, Kou J, Zhu D, Yu B. Comparison of neuroprotective effects of flavonoids, terpenoids, and their combinations from *Ginkgo biloba* on ischemia reperfusion- injured mice. Pharm Biol 2008;45:728-33.
54. Karolina A, Wojtunik K, Katarzyna TD, Katarzyna K, Grazyna G, Krzysztof J, *et al.* Volatile terpenoids as potential drug leads in Alzheimer's disease. Open Chem 2017;15:332-43.
55. Usmani S. Screening for antioxidant and free radical scavenging potential of extracts of leaves and flowers of *Calotropis gigantea*. Asian J Pharm Clin Res

2013;6:97-100.
56. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
57. Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem 2001;75:197-202.
58. Huie RE, Padmaja S. The reaction of no with superoxide. Free Radic Res Commun 1993;18:195-9.
59. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J Neurol Neurosurg Psychiatry 1999;66:137-47.
60. Lane RM, Potkin SG, Enz A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. Int J Neuropsychopharmacol 2006;9:101-24.
61. Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: Pharmacology and toxicology. Curr Neuropharmacol 2013;11:315-35.
62. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the cholinergic system. Curr Neuropharmacol 2016;14:101-15.