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FARMACIA, 2023, Vol. 71, 2
https://doi.org/10.31925/farmacia.2023.2.9 **ORIGINAL ARTICLE**

BRAIN ANTIOXIDANT PROPERTIES OF *PIPER CUBEBA* L. EXTRACTS AND ESSENTIAL OIL

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Abstract

This study aimed to discover the antioxidant properties of *Piper cubeba* extract and essential oil in rats' brains. Antioxidant activity of *P. cubeba*'s 96% ethanol extract (PC96), 70% ethanol extract (PC70), and essential oil (PO) was tested based on lipid peroxide, catalase (CAT), and superoxide dismutase (SOD) activities and nitric oxide (NO) concentration in the brain. The rats were given the samples for a week p.o, then the following day, the brain was collected. The metabolite content of the extract was analysed by Thin Layer Chromatography (TLC). The result showed that PC96, PC70, and PO could significantly inhibit the lipid peroxidase in rat's brain ($p < 0.01$). This activity was in line with the decrease in NO concentrations in all groups. However, there was no effect of *P. cubeba* on the SOD despite increased CAT activity. Overall, *P. cubeba* showed antioxidant activities in the brain, especially the 96% ethanol extract, therefore worth being developed as a neuroprotective agent.

Rezumat

În acest studiu ne-am propus evaluarea proprietăților antioxidante ale extractului de *Piper cubeba* și ale uleiului său esențial asupra țesutului cerebral de șobolan. Activitatea antioxidantă a extractului etanolic 96% (PC96), a extractului etanolic 70% (PC70) și a uleiului esențial (PO) de *P. cubeba* a fost testată pe baza activităților catalazei (CAT), a superoxid dismutazei (SOD), a oxidului nitric (NO) și a peroxidării lipidice din țesutul cerebral. Șobolanilor li s-au administrat preparatele timp de o săptămână p.o, după care le-a fost recoltat creierul. Conținutul de metaboliți al extractului a fost analizat prin cromatografie în strat subțire (TLC). Rezultatul a arătat că PC96, PC70 și PO ar putea inhiba semnificativ peroxidarea lipidică din creierul șobolanului ($p < 0,01$). Această activitate a fost în concordanță cu scăderea concentrațiilor de NO în toate grupurile. Cu toate acestea, nu a existat niciun efect al *P. cubeba* asupra SOD, în ciuda activității CAT crescute. În general, *P. cubeba* a prezentat activități antioxidante la nivel cerebral, în special pentru extractul etanolic 96%.

Keywords: Brain, antioxidant, *Piper cubeba*, SOD, CAT, MDA, NO

Introduction

Under normal conditions, reactive oxygen species (ROS) in brain tissue, such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and peroxynitrite anion, are balanced by some endogenous enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPX), and catalase (CAT) as well as non-enzymatic mechanism such as glutathione, uric acid, vitamins C and E as antioxidants. SOD is an initial defence against oxidative stress by catalysing the dismutation reaction of superoxide anions to hydrogen peroxide, while GPX and CAT further protect cells by converting hydrogen peroxide to water. However, in some cases, such as cerebral ischemia, free radicals significantly increase and overpower endogenous antioxidants, causing brain damage [24].

Neuroprotection is a strategy to protect the brain from acute injury and chronic disorders [21]. Many potential antioxidants have been developed for neuroprotective purposes, such as in Alzheimer's [5], brain injury [16] and stroke [24]. A preliminary study on the antioxidant activity was usually done for neuroprotective agent screening [11, 14]. Due to the antioxidant activity, some plants have been studied for their neuroprotection activity, like eugenol, which was beneficial in traumatic brain injury [4]. Pretreatment using methanol extract of *Artemisia absinthium* L. has also been shown to have behavioral disturbances in rats induced by ischemic stroke [6]. Developments of nanomedicine neuroprotectors have also been carried out using natural ingredients, such as pomegranate oil in the form of nanoemulsions [27] and curcumin nanoemulsion for recovery of hemorrhagic stroke rats [25].

Piper cubeba (L.) (*Piperaceae*), known as cubeb or *kemukus* in Indonesia, have been studied for several activities, including anti-inflammatory [7], antioxidant [3], antimutagenic [34] and antiparasite [28]. Traditionally, *P. cubeba* is used for various diseases such as anti-asthmatic, anti-inflammatory [1], rheumatism, dysentery and gonorrhoea [18]. The compounds of this plant have been confirmed to reduce the hydroxyl radical,

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superoxide anion radical, and 2,2-diphenyl-1-picryl-hydrazyl radical [2]. The methanol extract showed increased plasma antioxidant activity after 21 days of administration at 200 mg/kg rats BW (bodyweight) [8]. The essential oil of this fruit also shows decent antioxidant properties [3, 31], and one of the compounds, cubebin, is shown to have the neuro-protective potential [32]. Even though studies of this fruit's antioxidant and neuroprotection activities have been established, the specific effect on the brain is very limited. Therefore, we tested the capacity of antioxidant activity of *P. cubeba* extracts and essential oil in the brain.

Materials and Methods

Plant Material

Dried *P. cubeba* fruits was obtained from Balai Penelitian Tanaman Rempah dan Obat (Balitro), Bogor, Indonesia. The fruit was then mashed and sieved using a 60 mesh to obtain a fine powder.

Animals

This research used 30 Wistar rats, 2 - 3 months old with a bodyweight of 175 - 200 g. The animals were divided into 6 groups (5 animals each): 96% ethanol extract (PC96) 200 and 400 mg/kg rats body weight (BW), 70% ethanol extract (PC70) 400 mg/kg, and essential oil (PO) 400 mg/kg, Vit-C (Vitamin C) 400 mg/kg, and N (control). The protocol was approved by the Health Research Ethics Commission, Muhammadiyah University, Jakarta, Indonesia. The animals were acclimatized in a cage at 23 ± 2°C for one week.

Preparation of Ethanol Extract

Dry *P. cubeba* fruit powder (1 kg) was extracted with 70 and 96% ethanol. The powder was extracted using a macerator in each solvent in a ratio of 1:5 for 24 hours. During the immersion, stirring was carried out for the first 6 hours and then continued occasionally. After one day, the extract was filtered, and the residue was re-macerated four times. The extract was then concentrated with a rotary evaporator at a temperature of 50°C, 60 rpm. Phytochemical screening was performed to determine the metabolites such as alkaloid, flavonoid, tannins, saponin and steroid/triterpenoid.

Essential Oil Extraction

A total of three kg of dried *P. cubeba* fruits powder were hydro-distilled for 3 hours using Clevenger-type apparatus. The obtained oil was added with anhydrous sodium sulfate to dry the moisture traces and then filtered.

Thin-layer chromatography (TLC)

Chemical contents of extract were checked by TLC with the following condition. The stationary phase was a silica gel 60 F_{254}, with chloroform: n-hexane (7:3) as the mobile phase. The extracts were spotted on a plate pack using a capillary tube and developed in a chamber (saturated with the mobile phase). Detection of components using 254 and 366 nm ultraviolet light. The plate is then sprayed with 10% sulfuric acid and heated to produce color.

Total phenolic and Flavonoid content

An amount of 0.5 mL of sample solution (1000 ppm in methanol) was added with 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 1 M sodium carbonate, then incubated for 15 minutes. The absorbance was measured using a UV-visible spectrophotometer at a wavelength of 765 nm with gallic acid as the standard for determining phenol content. While the flavonoid concentration was determined using quercetin as standard. 0.5 mL of sample solution (1000 ppm in methanol) was added with 1.5 mL of methanol, 2.8 mL of distilled water, 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1 M sodium acetate. The mixtures were incubated for 30 minutes, and the absorbance was measured at 415 nm.

Brain homogenate preparation

The animals were given the test substance p.o for a week. The next day, the rats were euthanized, and then the brains were collected. Brain homogenate was prepared immediately at 20% concentration in PBS (phosphate-buffered saline). PBS (pH 7.5) was previously added with 0.1 M of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF). The brain was homogenised on an ice bath and then centrifuged at 4000x g, 4°C for 20 minutes. Homogenate was stored at -20°C until testing. Total protein content was determined by spectrophotometer UV-Vis using BSA as a standard. 30 µL of brain homogenate was added with 1.5 mL Bradford reagent and incubated for 5 minutes. The absorbance was read at 595 nm.

SOD activity test

SOD activity was determined using the Total SOD (T-SOD) activity assay kit (Elabscience®). A total of 20 µL of diluted brain homogenate (dilution factor 100) was used for the test. 50% SOD inhibition is equivalent to 1 SOD activity unit (U).

$$i = \frac{(Acontrol - Ablank\ control) - (Asample - Ablank\ control)}{(Acontrol - Ablank\ control)} \times 100\%$$

T-SOD is calculated using the following equation:

$$T - SOD\ activity\ (U/mgprot) = i \div 50\% \times \frac{V1}{V2} \times f \div Cpr$$

i (inhibition ration of SOD), V1 (total volume of reaction), V2 (sample volume), f (dilution factor), and Cpr (protein concentration mg protein/mL).

Catalase (CAT) Activity Test

The procedure was done following existing research [14]. The CAT activity was determined using the equation:

$$\text{CAT activity (U/gram)} = \left(\frac{2.393}{t} \log \frac{S0}{S}\right)/f$$

t (incubation time, which is 2 minutes), S0 (standard absorbance), S (sample absorbance) and f (dilution factor).

Nitric Oxide (NO) Determination
The procedure was carried out following existing research with slight modification [32]. 200 µL of the sample was added with 200 µL of 2 N $KMnO_4$ and allowed to stand for 30 minutes. Then 800 µL of 5% (v/v) salicylic acid in H_2SO_4 was added and waited for 20 minutes. Finally, 8 N NaOH was added to 10 mL. The mixture was then filtered, and the absorbance was measured by a spectrophotometer at 414 nm.
Lipid Peroxidation Test
An amount of 250 µL of brain homogenate was added with 250 µL 20% TCA and 0,5 mL 0,67% TBA, then heated at 100°C for 10 minutes. The mixture was allowed to cool in an ice bath and then centrifuged. The absorbance was measured at 532 nm with TEP as standard. One microliter of 97% TEP was added with distilled water up to 100 mL to obtain a 4.05 nmol/mL TEP solution.
Statistic analysis
The data were tested for homogeneity and normality, followed by a one-way ANOVA test with a significance of 95%.

Results and Discussion

Phytochemical Profile
Phytochemical screening showed that PC96 contained flavonoids, tannins, quinones and steroids, while PC70 contained the same compounds except for steroids. This study confirmed the presence of lignans in the extracts by thin-layer chromatography (TLC), which is the dominant compound of the fruits [12]. The results showed slightly different spots between PC70 and PC96. Sulfuric acid was used as a staining reagent for lignans, resulting in various color bands in both extracts. It could also be observed that there are more non-polar compounds in the PC96 than in PC70.
In addition, the phenol and flavonoid content of the two extracts were also determined. The results showed that PC70 has higher phenol content while PC 96 has higher flavonoid content (Table I).

Table I
Total yields, phenol, and flavonoid of 96% ethanol, 70% ethanol, and essential oil of *Piper cubeba*

Test substance	Yields (%)	Phenol (mg GAE/g extract)	Flavonoid (mg QE/g extract)
PC96	13.7	101.34 ± 1.43	215.02 ± 3.27
PC70	14.89	206.99 ± 4.4	146.86 ± 6.43
PO	0.2	-	-

Antioxidant activity of Piper cubeba
Natural products have shown neuroprotective potential through anti-inflammatory, immunomodulatory, and antioxidant mechanisms [20]. Screening of antioxidant activity, especially in the brain, is the first step in the algorithm of neuroprotective agents research [23].
The brain is one of the organs vulnerable to oxidative stress because this organ contains high lipids, low enzymatic antioxidants, and high oxygen consumption [29]. Reactive oxygen species (ROS) could be produced in the brain through the mitochondrial respiratory chain (MRC), nicotinamide adenine dinucleotide phosphate (NAPDH) oxidation, and xanthine oxidase [26]. Mitochondrial superoxide is a by-product of ATP generation. This superoxide will be converted by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2) before leaving the mitochondria [30]. H_2O_2 will then react with catalase (CAT) and be converted into H_2O and O_2. ROS could also react with nitric oxide synthase (NOS) to produce nitric oxide ($NO^•$) radicals, which further react with ROS to form peroxynitrite radicals ($ONOO^-$) [13]. In this study, the antioxidant activities of *P. cubeba* were determined based on the SOD and CAT activity, changes in NO concentration and lipid peroxidation inhibition (Figure 1).
SOD is widely excreted in various organs, but its levels were found to be low in the brain [10]. SOD levels can be influenced by several things, such as the type of SOD isoform, the length of time cells are exposed to oxidative reactions and the proportion of free radicals. A study of the antioxidant activity of *P. cubeba* at a dose of 200 mg/kg rats BW showed an increase in SOD activity after two weeks of oral administration [8], while in this study, SOD levels were lower than control after administration of *P. cubeba*, except for the PO group (Figure 1a). Further research is necessary to determine the effect of *P. cubeba* on SOD activity with various doses and administration times. The Vitamin C group also showed lower SOD activity than the control, which is in line with another study, where Vitamin C resulted in inhibition of all SOD isoforms in the rat's brain [19].
Despite the decrease in SOD activity, there was an increase in CAT activity after *P. cubeba* administration. Figure 1b depicts that *P. cubeba* could significantly increase CAT activity, except for the P70 group. This study also examined the concentration of NO, which is presented by $NaNO_2$ [33]. NO could directly become the source of oxidative stress, and NO could also react with ROS to produce a peroxynitrite oxidant, which can cause an increase in lipid peroxidation and ultimately damage DNA [30]. In this study, the administration of the extracts and essential oil of *P. cubeba* significantly ($p < 0.05$) suppressed the NO (Figure 1c). This result was in line with the lipid

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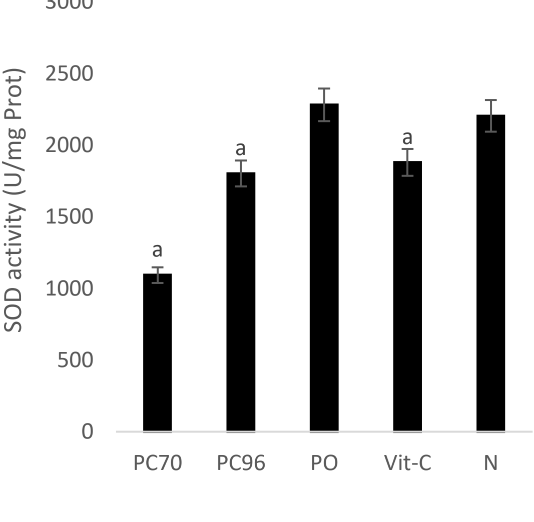
peroxidase test, where all test groups showed a lower MDA concentration significantly ($p < 0.01$) compared to N (Figure 1d).

The research also examined the antioxidant activity of PC96 in two doses (200 and 400 mg/kg). The results showed no significant difference in antioxidant effect between doses of 200 mg/kg and 400 mg/kg of PC96 (Figure 2), which might be because of the limited transport of the test substance to the brain.

Figure 3 illustrates the overall assumed mechanism of *P. cubeba* as an antioxidant in the brain. The extracts could increase CAT activity, reduce NO concentration and limit lipid peroxidation. *P. cubeba* contains several phytoconstituents known to have antioxidant activity, such as terpenoids (essential oils), flavonoids and lignans. Polyphenols have been known to exhibit potent antioxidant activity in plants [9, 17]. Polyphenols of *P. cubeba* include gallic acid, caffeic acid, and ferulic acid [1], as well as cubebin, hinokinin and yatein, the dominant lignans in *P. cubeba* [12]. Cubebin might contribute as one of the active components, where this compound has been proven as a neuroprotector in mice induced by scopolamine, one of which was through the anti-oxidant mechanism[32].



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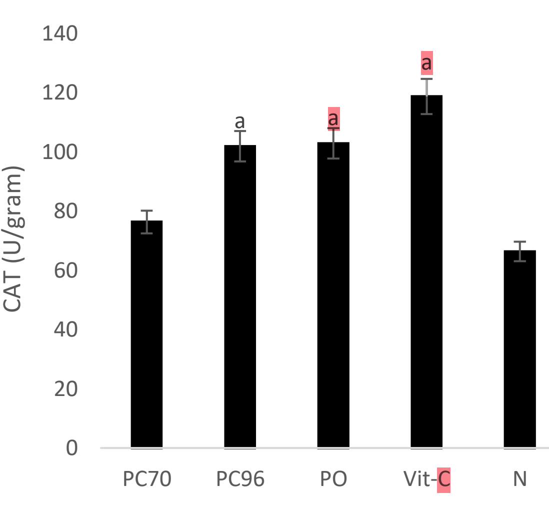

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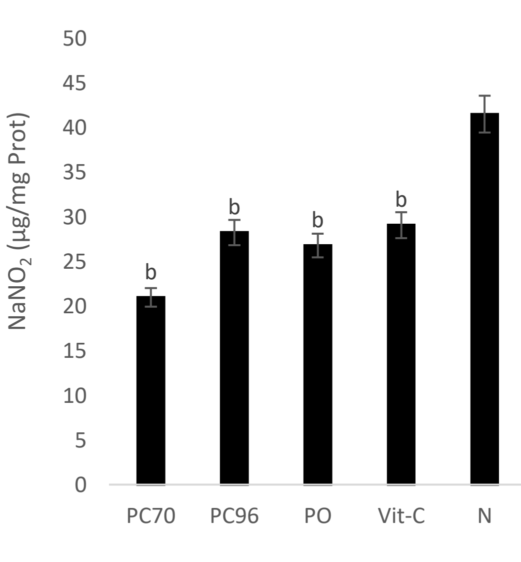

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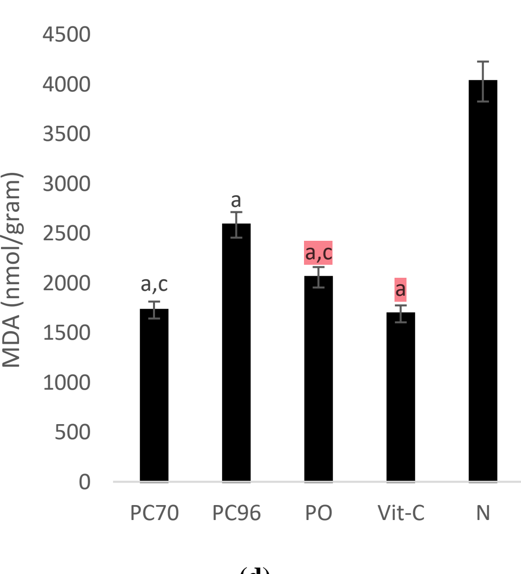

(d)

Figure 1.

The antioxidant effects of *P. cubeba* on rat's brain based on activities of SOD (a), CAT (b), the concentration of NO (c) and inhibition of Lipid peroxidation (d)

[a]significantly different to N at $p < 0.01$,

[b]significantly different to N at $p < 0.05$,

[c]comparable to Vit-C at $p > 0.05$

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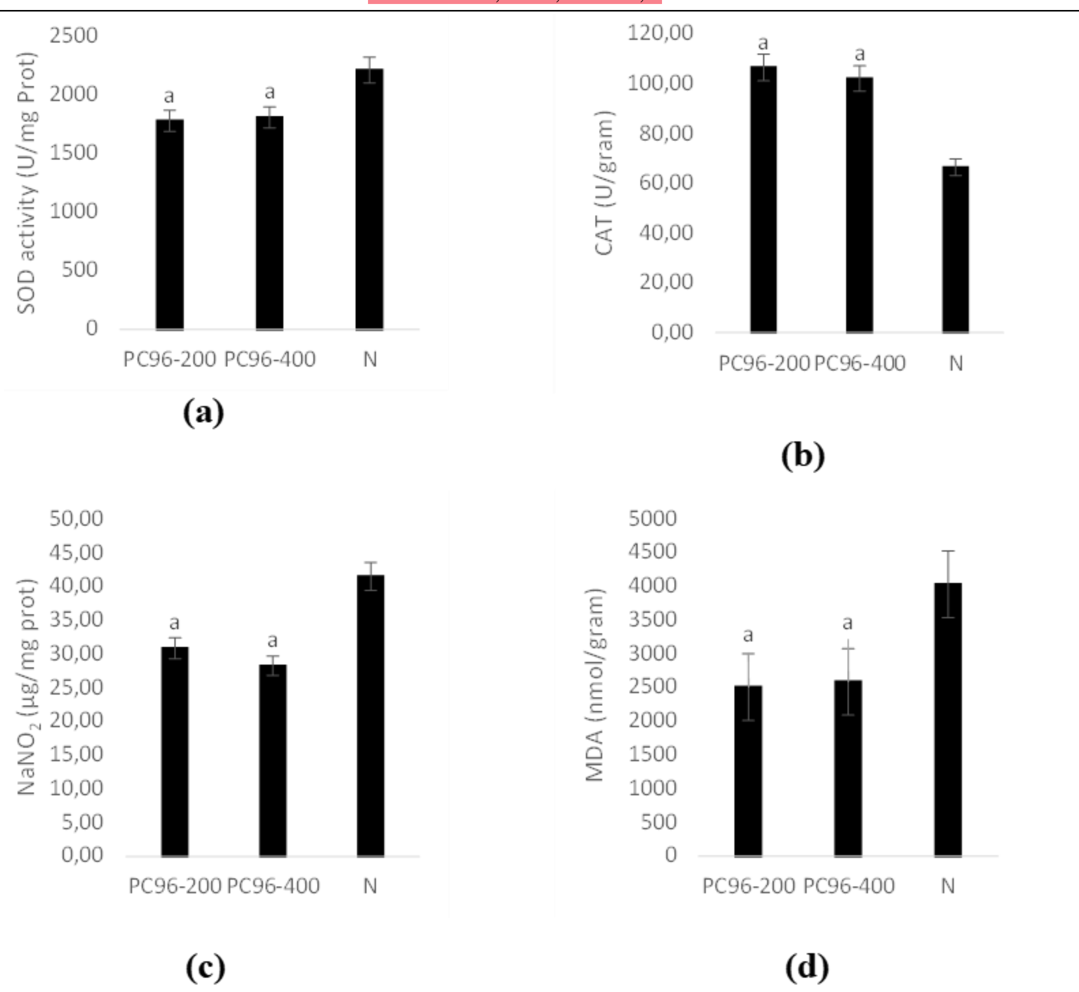

Figure 2.
The antioxidant effects of low and high doses of 96% ethanol extract of *P. cubeba* on rat's brain based on activities of SOD (a), CAT (b), the concentration of NO (c) and inhibition of Lipid peroxidation (d)
[a]significantly different to N at $p < 0.01$

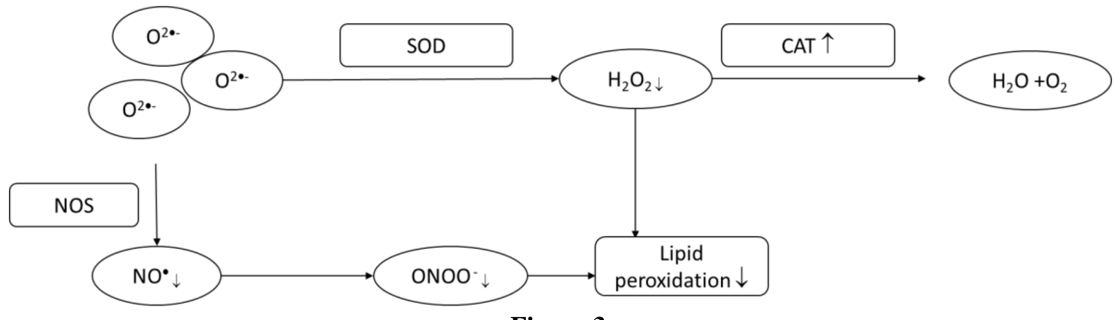

Figure 3.
Assumed mechanism of action of *Piper cubeba* as an antioxidant in the brain↑(increased) ↓(decreased)

Conclusions

This study proves the ability of *P. cubeba* as an antioxidant in the brain, both the extracts and essential oils. The best activity was shown by 96% ethanol extract of *P. cubeba*, which increased CAT activity by 60%, inhibited lipid peroxide by 37% and decreased NO production by 32% compared to normal control. This study underlies further research on *P. cubeba* as a neuroprotective agent.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Abdul-jalil TZ, Nasser ZA, *Piper cubeba*: Phytochemical and pharmacological review of a routinely used spices. *Int J Pharm Res.*, 2020; 12(Suppl Issue 1): 761-768.
2. Aboul-Enein HY, Kładna A, Kruk I, Radical scavenging ability of some compounds isolated from *Piper cubeba* towards free radicals. *Luminescence*, 2011; 26(3): 202-207.
3. Alminderej F, Bakari S, Almundarij TI, Snoussi M, Aouadi K, Kadri A, Antioxidant Activities of a New Chemotype of *Piper cubeba* L. Fruit Essential Oil (Methyleugenol/Eugenol): *In Silico* Molecular Docking and ADMET Studies. *Plants*, 2020; 9(11): 1534.
4. Barot J, Saxena B, Therapeutic effects of eugenol in a rat model of traumatic brain injury: A behavioral, biochemical, and histological study. *J Tradit Complement Med.*, 2021; 11(4): 318-327.
5. Behl C, Moosmann B, Antioxidant Neuroprotection in Alzheimer's Disease as Preventive and Therapeutic Approach. *Free Radic Biol Med.*, 2002; 33(2): 182-191.
6. Bora KS, Sharma A, Neuroprotective effect of *Artemisia absinthium* L. on focal ischemia and reperfusion-induced cerebral injury. *J Ethnopharmacol.*, 2010; 129(3): 403-409.
7. Choi EM, Hwang JK, Investigations of anti-inflammatory and antinociceptive activities of *Piper cubeba*, *Physalis angulata* and *Rosa hybrida*. *J Ethnopharmacol.*, 2003; 89(1): 171-175.
8. Choi EMM, Hwang JKK, Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats. *Phytother Res.,* 2005;19(5):382-386.
9. Costea T, Bădiceanu CD, Nuță DC, Gîrd CE, Limban C, Karampelas O, Antioxidant activity and drug profile of several thiourea derivatives of 2-thiophene carboxylic acid. *Farmacia*, 2020; 68(6): 1055-1061.
10. Davis SM, Pennypacker KR, Targeting antioxidant enzyme expression as a therapeutic strategy for ischemic stroke. *Neurochem Int.*, 2017; 107: 23-32.
11. Drăgan F, Moisa CF, Teodorescu A, Burlou-Nagy C, Fodor KI, Marcu F, Popa DE, Teaha DIM, Evaluating *in vitro* antibacterial and antioxidant properties of *Origanum vulgare* volatile oil, *Farmacia*, 2022; 70(6): 1114-1122.
12. Elfahmi, Ruslan K, Batterman S, Bos R, Kayser O, Woerdenbag HJ, Lignan profile of *Piper cubeba*, an Indonesian medicinal plant. *Biochem Syst Ecol.*, 2007; 35(7): 397-402.
13. Fernando V, Zheng X, Walia Y, Sharma V, Letson J, Furuta S, S-Nitrosylation: An Emerging Paradigm of Redox Signaling. *Antioxidants*, 2019; 8(9): 404.
14. Nicolae AC, Arsene AL, Vuță V, Popa DE, Sîrbu CA, Burcea Dragomiroiu GTA, Dumitrescu IB, Velescu BȘ, Gofiță E, Drăgoi CM, *In vitro* P-GP expression after administration of CNS active drugs. *Farmacia*, 2016; 64(6): 844-850.
15. Hadwan MH, Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC Biochem.*, 2018; 19(1): 1-8.
16. Hall ED, Wang JA, Miller DM, Cebak JE, Hill RL, Newer pharmacological approaches for antioxidant neuroprotection in traumatic brain injury. *Neuropharmacol.*, 2019; 145(Pt B): 247-258.
17. Humulescu I, Flutur MM, Cioanca O, Mircea C, Robu S, Marin-Batir D, Spac A, Corciova A, Hancianu M, Comparative chemical and biological activity of selective herbal extracts. *Farmacia*, 2021; 69(5): 861-866.
18. Jamal JA, Ghafar ZA, Husain K, Medicinal Plants used for Postnatal Care in Malay Traditional Medicine in the Peninsular Malaysia. *Pharmacogn J.*, 2011; 3(24): 15-24.
19. Kao PF, Lee WS, Liu JC, Tsai JC, Hsu YH, Chang WY, Cheng TH, Liao SS, Downregulation of superoxide dismutase activity and gene expression in cultured rat brain astrocytes after incubation with vitamin C. *Pharmacology*, 2003; 117(111): 1-6.
20. Kim YC, Neuroprotective phenolics in medicinal plants. *Arch Pharm Res.*, 2010; 33(10): 1611-1632.
21. Kumar GP, Khanum F, Neuroprotective potential of phytochemicals. *Pharmacogn Rev.*, 2012; 6(12): 81-90.
22. Lima TC, Lucarini R, Volpe AC, de Andrade CQJJ, Souza AMPP, Pauletti PM, *In vivo* and *in silico* anti-inflammatory mechanism of action of the semisynthetic (−)-cubebin derivatives (−)-hinokinin and (−)-O-benzylcubebin. *Bioorg Med Chem Lett.*, 2017; 27(2): 176-179.
23. Liu W, Ma H, DaSilva NA, Rose KN, Johnson SL, Zhang L, Development of a neuroprotective potential algorithm for medicinal plants. *Neurochem Int.*, 2016; 100: 164-177.
24. Margaill I, Plotkine M, Lerouet D, Antioxidant strategies in the treatment of stroke. *Free Radic Biol Med.*, 2005; 39(4):429-443.
25. Marques MS, Cordeiro MF, Marinho MAGG, Vian CO, Vaz GR, Alves BS, Curcumin-loaded nanoemulsion improves haemorrhagic stroke recovery in Wistar rats. *Brain Res.*, 2020; 1746: 147007.
26. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB, Reactive oxygen species in inflammation and tissue injury. *Antioxidants Redox Signal.*, 2014; 20(7): 1126-1167.
27. Mizrahi M, Friedman-Levi Y, Larush L, Frid K, Binyamin O, Dori D, Pomegranate seed oil nanoemulsions for the prevention and treatment of neurodegenerative diseases: The case of genetic CJD. *Nanomedicine*, 2014; 10(6): 1353-1363.
28. Parreira RLT, Costa ES, Heleno VCG, Magalhães LG, Souza JM, Pauletti PM, Evaluation of lignans from *Piper cubeba* against *Schistosoma mansoni* adult worms: a combined experimental and theoretical study. *Chem Biodivers.*, 2019; 16(1): e1800305.
29. Prasad N, Ramteke P, Dholia N, Yadav UC, Chapter 27. Therapeutic Interventions to Block Oxidative Stress-Associated Pathologies. Immunity and Inflammation in Health and Disease, Chatterjee S, Jungraithmayr W, Bagchi D, Elsevier Inc. US, 2018: 341-342.
30. Shirle R, Ord ENJ, Work LM, Oxidative stress and the use of antioxidants in stroke. *Antioxidants*, 2014; 3(3): 472-501.

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Submission ID trn:oid:::1:3028239784

31. Singh G, Marimuthu P, Heluani CSD, Catalan CAN, Chemical constituents, antioxidative and antimicrobial activities of essential oil and oleoresin of tailed pepper (*Piper Cubeba* L). *Int J Food Eng.*, 2007; 3(6): 11.
32. Somani GS, Nahire MS, Parikh AD, Mulik MB, Ghumatkar PJ, Laddha KS, Neuroprotective effect of Cubebin: A dibenzylbutyrolactone lignan on scopolamine-induced amnesia in mice. *Indian J Med Res.*, 2017; 146(2): 255-259.
33. Termin A, Hoffman M, Bing R, A simplified method for the determination of nitric oxide in biological solution. *Life Sci.*, 1992; 51: 1621-1629.
34. Zahin M, Khan MS, Abul Qais F, Abulreesh HH, Ahmad I, Antioxidant properties and anti-mutagenic potential of *Piper Cubeba* fruit extract and molecular docking of certain bioactive compounds. *Drug Chem Toxicol.*, 2018; 41(3): 358-367.

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