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Antioxidant Activity of Different Parts of *Nauclea subdita*

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ABSTRACT

Nauclea subdita (Taya) has been used empirically to treat hypertension patients in Kalimantan Island. Recent studies reported that hypertension is very closely related to oxidative stress. Natural antioxidants from plants act as radical scavengers by converting free radicals into less reactive substances. Therefore, a plant possessing antioxidant activity could be a promising agent as an alternative treatment for hypertension. This study aimed to determine the *in vitro* antioxidant activity of aqueous extract of the stem bark and leaves of *Nauclea subdita*. Assay methods used in this study were Ferric Reducing Antioxidant Power (FRAP), 2.2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). Furthermore, a molecular docking study was performed to predict the potential antioxidant activity of several chemical constituents of the plant against protein kinase C. The results indicated that the antioxidant activity of Taya extracts increases linearly with its concentration, as also indicated by DPPH and ABTS assay. According to the ABTS assay, the stem bark and leaves extract possessed antioxidant activity with IC_{50} values of 1183.13 ppm and 116.42 ppm, respectively and 128.63 ppm and 26.95 ppm for DPPH assay. At the same time, the FRAP assay showed ferric reducing power, which correlates to its antioxidant activity. The Molecular docking study showed that two metabolites from the phytosteroid class (*β*-sitosterol and stigmast-4-en-3-one) yielded comparable activity with native ligands. Aqueous extract of Taya stem bark and leaves possess antioxidant capacity, which could be further studied as a potential agent to reduce oxidative stress in hypertension.

***Keywords*:** Taya, Antioxidant, DPPH, ABTS, FRAP.

Introduction

Antioxidants are commonly found in natural ingredients, several health supplements, and the body. Antioxidants play an essential role as health protecting agents by inhibition of oxidative stress.[1] Antioxidant agent can protect the human body from harmful free radicals, which increases the risk of developing several metabolic diseases such as diabetes, hypertension, cancer, and other physiological disorder.[2] Natural antioxidants from plants such as carotenoids, lycopene, *β*-carotenes, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites act as radical scavengers by converting free radicals into less reactive chemicals.[3]Free radicals induced cell damage to have been implicated in the ageing processes and other degenerative diseases.[4] Currently, a new hypothesis is evolving, suggesting that oxidative stress reactions play an essential role in the pathogenesis of hypertension.[5] Oxidative stress can promote cell damage by post-translational modification of proteins.[6] Hypertension is a major risk factor contributing to other physiological disorders, such as heart failure, myocardial infarction, stroke, peripheral arterial disease and chronic kidney disease. During hypertension, an increase in oxidative stress was observed.[7] Many reports also described that antioxidants have an improvement role in many oxidative stress disorders such as Atherosclerosis, Diabetes, Alzheimer, Parkinson, and HIV Infection.[8]

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Increased production of hydrogen peroxide and lipid hydroperoxide could be found in hypertensive patients. A reduction in the activity of superoxide dismutase and glutathione peroxidase has been found in newly diagnosed hypertensive patients. These enzymes are inversely proportional to blood pressure. Again, a decrease in catalase and superoxide dismutase levels and increased reactive oxygen species (ROS) or reactive nitrogen species (RNS) contribute to oxidative stress. Excessive reactive oxygen species (ROS) can become the cause of or worsen hypertension.[5] The pathophysiology explained above illustrates the vital role of antioxidants in hypertension. Antioxidants could be used as the defence system to neutralize excess levels of reactive oxygen in hypertension. Therefore, it has the potential to be used as adjunctive therapy in hypertensive patients.[9]

Taya (*Nauclea subdita*) is Indonesia's native plant from Borneo, used empirically for diabetes, stomach ache, skin diseases, and hypertension therapy. A previous study demonstrated that ethanolic stem bark extract of *N. subdita* possessed high antioxidant activity with an IC_{50} value of 48.78 µg/mL, categorized as a potent antioxidant with IC_{50} values <50 µg / mL.[10,11] Another study reported that angustin and nucleofin, compounds found in Taya were active as vasodilators.[12] In this study, the aqueous extract was used to enhance safety. This study aimed to determine the antioxidant activity of aqueous extract of *N. subdita* stem bark and leaves. *In vitro*, antioxidant activity was evaluated using Ferric Reducing Antioxidant Power (FRAP); 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS). In addition, a molecular docking study was performed against protein kinase C β II (35) to predict the possible antioxidant mechanism via the PKC pathway.[1]

Materials and Methods

Plant material

Nauclea subdita (Korth.) Steud was collected from Katingan, Central Kalimantan, Indonesia, in March 2019. The plant was authenticated

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and deposited at the herbarium of the Center for Information and Development of Traditional Medicine (PIPOT) at Universitas Surabaya, Surabaya, Indonesia with voucher number 1368/D.T/I/2019.

Preparation of extract
The stem barks and leaves were thoroughly washed using fresh tap water and subsequently air-dried. The dried stem bark was powdered using a mechanical grinder, and leaves were cut into small parts. The samples were extracted separately by refluxing 100 g of each (stem bark and leaves) with 300 mL demineralized water for 2 hours. This step was repeated three times to achieve total extraction. The extract was filtered, and the filtrate was freeze-dried. The dried extract was stored in a desiccator until further use.

Other materials
Other materials used in this study include gallic acid, glacial acetic acid, iron (III) chloride hexahydrate, potassium persulfate, aluminium chloride (Merck, Darmstadt, Germany), TPTZ, ascorbic acid, sodium acetate trihydrate (Himedia, Mumbai, India), 2.2-diphenyl-1-picrylhydrazyl (DPPH), 2.2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and quercetin(Sigma-Aldrich Pte. Ltd., Singapore), demineralized water, ethanol, HCl, Folin-Ciocalteu reagent, 7.5% sodium carbonate (Merck. Darmstadt. Germany), 5% sodium nitrite (Merck, Darmstadt, Germany), 10% aluminium chloride (Merck, Darmstadt, Germany), 1 M sodium hydroxide (Merck, Darmstadt, Germany).

Phytochemical screening
Phytochemical screening was performed to identify essential oils, alkaloids, flavonoids, polyphenols, and saponins present in the samples.

Determination of total phenolic content
Folin Ciocalteu's method was used to determine the total phenolic content of the extracts. Gallic acid at various concentrations (2, 4, 6, 8 and 10 ppm) was used as a standard to obtain a calibration curve. The sample (1 mL) was pipetted into a volumetric flask, and 500 µL of Folin-Ciocalteu reagent was added. It was then shaken and left for 8 minutes, then 4 mL of 7.5% Na_2CO_3 and solvent was added to each sample to obtain a final volume of 10 mL. The absorbance of the extracts was measured at the wavelength of 750nm. The total phenolic content of the extracts was expressed as µg/mg of gallic acid equivalent (GAE/mg).[14]

Determination of total flavonoid content
The aluminium chloride method was used to determine the total flavonoid content of the extract. Quercetin at various concentrations (5, 10, 15, 20 and 25 ppm) was used as standard solutions to generate a calibration curve. 2 mL of each stem bark and leaves extract was pipetted into a volumetric flask. Four mL of demineralized water and 0.3 mL of 5% $NaNO_2$ were added and allowed to stand for 5 minutes, followed by 0.3 mL of 10% $AlCl_3$ solution. The mixture was left to stand for 1 minute. Two mL of 1 M NaOH was then added, and the volume of each sample was adjusted with demineralized water to a final volume of 10 mL. Absorbance was measured at a wavelength of 514 nm. The total flavonoid content of each aqueous extract was expressed as µg/mg of quercetin equivalent (QE/mg)).[14]

Antioxidant activity assay assessment using FRAP Method
The FRAP antioxidant assay method described by Morales & Pardedes and modified by Setiawan *et al*. was used to measure ferric tripyridyltriazine to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) iron reduction by aqueous extract of *N subdita* stem bark and leaves.[15.16] The FRAP reagent comprises 25 mL of a buffer solution with a pH of 3.6, TPTZ (2.5 mL) solution (156 mg was dissolved with 40 mmol/L HCl and made up to 50 mL), and 2.5 mL of iron (iii) chloride hexahydrate solution (540 mg/100 mL), mixed. Finally, a sufficient quantity of demineralized water was added up to 100 mL. FRAP reagent (100 µL) was added to 100 µL of the sample in a microplate. It was then incubated at 37° C for 10 minutes. Absorbance was measured at a wavelength of 593 nm. The FRAP value was quantified as Vitamin C equivalent.

Antioxidant activity assay using DPPH Method
The DPPH assay method described by Blois and modified by Setiawan *et al*. was used to determine the antioxidant activity of the plant samples. The sample (50 µL) was pipetted, and 150 µL of 300 ppm DPPH was added to the microplate.[15.17.18] The solution mixture was incubated at 37°C for 30 minutes. Absorbance was measured at a wavelength of 516 nm. The following equation was used to determine the % of inhibition:

$$\% \text{ inhibition} = \frac{absorbance\ (control - sample)}{absorbance\ (control)}\ x\ 100\%$$

Antioxidant assessment using ABTS method
The ABTS assay method described by Re *et al*. with some modifications was used to determine the antioxidant activity of the samples.[19] ABTS Reagent was prepared from 19.2 mg ABTS powder dissolved in demineralized water up to 5 mL, 3.31 mg of potassium persulfate was dissolved in demineralized water up to 5 mL. ABTS solution and potassium persulfate solution were left for 12 hours in the dark condition. ABTS solution and Potassium Persulfate solution were mixed in a 25 mL volumetric flask, and demineralized water was added. ABTS reagent 160 µL was mixed with 40 µl of sample, and the absorbance was measured at a wavelength of 734 nm.[14] The following equation determined the % inhibition:

$$\% \text{ inhibition} = \frac{absorbance\ (control - sample)}{absorbance\ (control)}\ x\ 100\%$$

Molecular docking study
The study was conducted against several chemical constituents found in the plant. Using Autodock 4.2.[14.20] All ligands were prepared by addition of Gasteiger partial charge.[21] Protein Kinase II, β C structure, was obtained from Protein Data Bank (PDB ID: 2I0E) and prepared by addition of hydrogen atom and Kollman partial charge.[22.23] Validity of the procedure was ensured by self-docking of the native ligand into its receptor. Lamarckian Genetic Algorithm was applied as the searching algorithm to obtain the best possible conformation for each compound, which was then evaluated compared to the native ligand.

Statistical analysis
All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation. Antioxidant activity was calculated by linear regression analysis to determine the equation $y = a + bx$; where the x-axis is concentration, and the y-axis is % value of inhibition.[18] IC_{50} values were determined by interpolation to calculate the concentration that inhibits half of the free radicals.

Results and Discussion

The extraction process of the stem bark and leaves of *N. subdita* was achieved by refluxing with water as a solvent. The extracted material was dried by freeze-drying. The extraction yield obtained was 6.11% for the stem bark and 33.33% for the leaves. This method was chosen based on an empirical approach in the Central Kalimantan community familiar with boiling *N. subdita* in water. The phytochemical screening of the aqueous extract of *N. subdita* stem bark and leaves indicated flavonoids, alkaloids, polyphenols in the leaves and saponins in the stem bark. Polyphenols, flavonoids, and alkaloids are potential compounds with antioxidant activity that play an essential role in controlling blood pressure and managing cardiovascular disease.[24.25] Liew *et al.* (2014) reported 12 new isolated compounds from the bark of *N subdita*, which were stigmast-4-en-3-one, *β*-sitosterol, naucleactonin C, benzamide, cinnamide, 1,2,3,4-tetrahydro-1-oxo-β-carboline, angustine, angustidine, nauclefine, harmane and angustolin.[13]
The determination of total phenolic and flavonoid contents in *N. subdita* stem bark and leaf was done by measuring the absorbance of a solution in a spectrophotometer. The result of total phenolic content in

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the stem bark and leaves are shown in Table 1. Total phenolic content measurement utilized gallic acid as standard, while flavonoid content measurement used quercetin. Plant phenolic compounds exist in various chemical scaffolds such as phenolic acids, flavonoids, tannins, stilbenes and lignans. Several pharmacological activities have been associated with the phenolic compounds, one of which is antioxidant because of its capability to transfer atom H from the OH group.[26]

Antioxidant capacity methods are generally divided into hydrogen transfer reaction (HAT) and Electron transfer reaction (ET). In this study, three different methods were employed to determine the mechanism of antioxidant activity. We used FRAP and DPPH methods to assess electron transfer reaction, while ABTS to assess hydrogen transfer mechanism. [27]

FRAP assay is one antioxidant assay that is considerably simple, stable, and highly reproducible. The determination of antioxidant activity using this method can be adequately executed if performed on antioxidant compounds that can reduce ferric-tripyridyltriazine (Fe(III)TPTZ) to ferrous-tripyridyltriazine (Fe(II)TPTZ) complex.[28,29]

Table 2 shows the results of antioxidant activity assessment using the FRAP method. This method was based on the reduction capacity mechanism from Ferric to Ferrous ion. The increased power of reduction was characterized by an increase in the amount of Ferrous ion formed. Reduction power was measured by comparing its capacity to vitamin C as a standard antioxidant. The smaller the equivalence of extract concentration to vitamin C, the higher the sample's reduction capacity, indicating the increase in sample capacity to electron transfer reaction as neutralized free radical.[9] Table 2 showed that *N. subdita* stem bark (50 ppm) extract provided a smaller equivalence to vitamin C than the leaves at 60 ppm. Hence it showed that *N. subdita* stem bark has better reducing power compared to *N. subdita* leaves extract.

DPPH is organic nitrogen radical which has a dark purple colour. This method is very popular in screening for natural antioxidants.[27] In principle, the DPPH free radical is reduced by the antioxidant species in the plant sample to non-radical (diphenyl picrylhydrazyl) compounds characterized by a change of colour from purple to yellow.[30] DPPH antioxidant assay can be evaluated by electron spin resonance or by a decrease of absorbance at wavelength 515-528 nm. Brand-William first reported this assay in 1995. The 50% inhibitory concentration (IC_{50}) is used to determine the antioxidant capacity of an extract, which means that smaller values give higher capacity.[31]

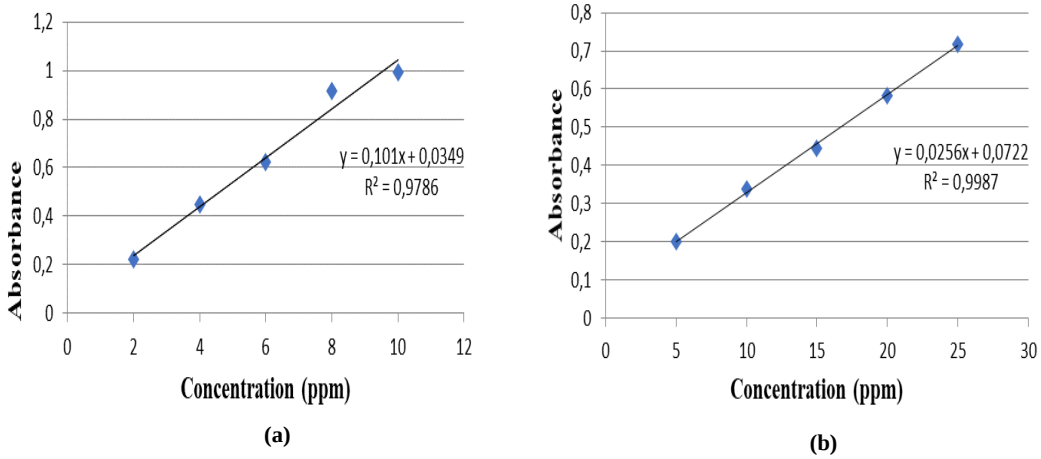
The results of antioxidant activity assessment using this method are depicted in Table 3. In this study, Vitamin C was used as a standard. Taya stem bark extract, leaves extract, and vitamin C had IC_{50} values of 1183.13 ppm, 116.42 ppm, and 31.60 ppm. According to this assay, the leaves extract had better antioxidant activity than the stem bark.

Increased Taya stem bark and leaf extract concentration also indicated a linear increase in inhibitory activity against DPPH radicals. This condition correlates to the different polyphenol and flavonoid contents in the plant samples (Table 1).

The principle of antioxidant activity assessment with the ABTS method is dependent on the antioxidant compound ability to stabilize free radicals by donating protons seen by changes in colour from blue to colourless of the ABTS solution.[29] ABTS is a nitrogen-centred radical with a blue-green colour, which changes to a colourless non-radical form when reduced by antioxidants. The ABTS method is very sensitive to light; even ABTS formation requires an incubation period of 12-16 hours in dark conditions.

The results of antioxidant assessment using the ABTS method are depicted in table 4. The inhibitory activity of Taya stem bark extract against ABTS radicals indicated linearity between concentration and inhibitory activity. Taya stem bark extract showed relatively more potent inhibitory activity in ABTS than in DPPH radicals. Extract inhibitory activity was demonstrated by IC_{50} value of 128.63 ppm, while that of vitamin C was 11.89 ppm. The leaves extract of Taya showed significant antioxidant activity with an IC_{50} value of 26.95 ppm. FRAP, DPPH and ABTS were different in reaction, therefore providing different antioxidant mechanisms with different results.[8]

The antioxidants present in the stem bark and leaves of Taya could be responsible for its antihypertensive activities and could help manage patients with hypertension. Phenol and flavonoid contents in Taya are believed to play a role in antioxidant activity because they have been reported to scavenge free radicals by donating protons from their hydroxyl groups.[31,32,33] Phenols are known to reduce the rate of oxidation by transferring the H atom (from the OH group) to the ROO chain of free radicals. Small molecule compounds and phenolic can reduce ABTS radical level, with a positive linear correlation of antioxidant capacity as concentration increases.[34] It is widely known that flavonoids such as polyhydroxy flavones, flavanones, flavanols, isoflavones, and chalcone possess high antioxidant capacity.[9]



(a) **(b)**

Figure 1: Calibration curve for phenolic and flavonoid content (a) galic acid and (b) quersetin

Table 1: Phenol and Flavonoid Content Determination in Taya (*Nauclea subdita*) Water Extract

No	Sample	Phenol Content (% GAE)	Flavonoid Content (% QE))
1	Taya Stem Bark Water Extract	4.22 ± 0.221	5.51 ± 0.380
2	Taya Leaves Water Extract	13.70 ± 0.715	6.16 ± 0.118

Note: GAE= Gallic Acid Equivalent; QE= Quercetin Equivalent

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Table 2: Antioxidant Activity of Taya Stem Bark and Taya Leaves Aqueous Extract Using FRAP (Ferric Reducing Antioxidant Power) Method

Sample	Concentration (ppm)	Absorbance ± SD
Ascorbic Acid	1.25	0.0553 ± 0.004
	2.5	0.1105 ± 0.004
	10	0.2358 ± 0.006
	20	0.5803 ± 0.009
	30	0.5810 ± 0.045
	40	0.9945 ± 0.038
Sample	**Concentration (ppm)**	**mg ascorbic acid / g sample ± SD**
Taya Stem Bark Water Extract	50	94.25 ± 1.626
	100	69.83 ± 2.544
	150	64.43 ± 3.039
	200	64.19 ± 2.252
	250	58.87 ± 2.011
Taya leaves Water Extract	20	550.583 ± 19.9682
	40	497.063 ± 16.9210
	60	502.197 ± 19.3950
	80	465.134 ± 15.4853

Table 3: Antioxidant Activity of Taya Stem Bark and Taya Leaves Aqueous Extract using DPPH (2.2-Diphenyl - picrylhydrazyl) Method

Sample	Concentration (ppm)	%Inhibition ± SD
Taya Stem Bark Water Extract	300	20.95 ± 2.166
	600	28.15 ± 1.593
	900	46.84 ± 1.079
	1200	51.43 ± 2.714
	1500	57.30 ± 3.465
IC_{50}		1183.13
Taya Leaves Water Extract	50	26.97 ± 3.688
	100	52.17 ± 0.979
	150	55.23 ± 3.249
	200	77.92 ± 0.431
	250	85.98 ± 0.739
IC_{50}		116.42
Ascorbic Acid	20	31.40 ± 1.376
	35	47.73 ± 2.031
	40	60.59 ± 1.034
	50	83.11 ± 2.299
IC_{50}		31.60

Table 4: Antioxidant Activity of Taya Stem Bark and Taya Leaves Aqueous Extract ABTS Method (2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

Sample	Concentration (ppm)	%Inhibition ± SD
Taya Stem Bark Water Extract	100	43.01 ± 2.206
	200	62.48 ± 4.535
	300	76.53 ± 1.958
	400	97.18 ± 0.9966
	500	102.52 ± 0.230
IC_{50}		128.63
Taya Leaves Water Extract	20	40.78 ±5.290
	30	54.24 ± 5.390
	40	66.63 ± 3.253
	50	86.591 ± 2.385
IC_{50}		26.05
Ascorbic Acid	10	37.86 ± 3.866
	15	56.45 ± 2.608
	20	74.90 ±2.259
	30	95.98 ± 2.307
	40	99.96 ± 0.226
IC_{50}		11.89

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Table 5: Molecular docking evaluation of the chemical constituents of *Nauclea subdita* against Protein Kinase C *β* II (PDB ID: 2I0E)

No	Compound	Docking Source	Amino Acid Interaction
1	Naucline	-8.88	Val 423
2	Nauclefine	-8.41	Val 423
3	Naucletine	-8.95	Val 423
4	Angustine	-8.59	Val 423
5	Angustoline	-8.59	Leu 348;Val 423
6	3.14-Dihydroangustoline	-8.97	Val 423
7	Angustidine	-8.36	Val 423
8	Subditine	-8.34	Val 423
9	Strictosamide	-8.30	Leu 348;Val 423;Asp 427
1	Pumiloside	-8.52	Leu 348;Asp 427
11	Naucleficine	-8.55	Val 423
12	Naucleactonin C	-8.73	Val 423
13	Harmane	-5.92	Thr 404
14	1.2.3.4-Tetrahydro-1-oxo-β-carboline	-5.86	Glu 390. Phe 485
15	Benzamide	-4.76	Thr 404. Glu 421
16	Cinnamide	-5.07	Thr 404
17	Bluemenol B	-6.37	Asp 484
18	Bluemenol A	-6.25	Asn 471; Asp 484
19	β-sitosterol	-10.00	Glu 390
20	Sigmast-4-en-3-one	-10.09	Phe 485
21	Vanillin	-3.83	Asp 484
22	Bisindolylmaleimide (native ligand)	-10.70	Thr 404; Glu 421; Val 423; Asp 470

Apart from the radical oxygen-based methods, molecular docking study was conducted to evaluate the antioxidant potentials of the constituents in *N. subdita* against the putative oxidant and antioxidant protein targets kinase C.[13] The results (Table 5) showed that only two metabolites from the phytosteroid class (*β*-sitosterol and stigmast-4-en-3-one) which yielded comparable activity against the native ligand. However, this result is not conclusive and should be elaborated further since cell-based assay results showed no significant correlation between phytosteroid compound and protein kinase C activity.[35]
The antioxidant power of Taya is expected to reduce the occurrence of oxidative stress in the body. Antioxidants are among the essential components in the metabolism and regulation of Reactive Oxygen Species (ROS), where ROS plays a critical role in the pathophysiology of hypertension. This is because an increase in ROS can reduce Nitric Oxide (NO) content in blood vessels and kidneys. In blood vessels, NO plays a vital role in blood regulation and vasodilatation.[35]Increased ROS in the kidney can trigger disruptions of sodium retention in the tubules, resulting in hypertension. Rodrigues *et al*. also point out that antioxidant therapy in hypertension is one of the approaches that can improve the quality of life.[36]

Conclusion

The study to determine in vitro antioxidant activity of aqueous extract of stem bark and leaves of *Nauclea subdita* has been done. DPPH and ABTS methods suggested that the aqueous leaves extract of *N. subdita* possess higher antioxidant activities than the aqueous stem bark extract. However, the FRAP method showed that the aqueous stem bark extract of *N subdita* have better reducing power activity than the leaves. Furthermore, the molecular docking results revealed that two phytosteroid phytoconstituents were responsible for the activity against Protein kinase C. However, further *in vitro* verification is necessary to validate this finding. Findings from this study have shown the potentials of *N. subdita* for use to manage oxidative stress-induced hypertension.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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The Tropical Journal of Natural Product Research is open access, peer-reviewed international journal aimed at making important contributions in the field of Natural Product Research, Pharmaceutical and Natural Sciences. The journal covers all aspects of Pharmaceutical research, chemistry and biochemistry of naturally occurring compounds, ethnomedicine, ethnopharmacology, pharmacognosy, biomedical research, biotechnology and related disciplines. The journal welcome submissions from a broad spectrum of scientific endeavour involving biological evaluation of natural substances of plant, microbial and animal origin against different disease targets, processes or therapeutic strategies that can lead to or assist in the prevention and management of chronic and infectious diseases, clinical therapeutics, Isolation and characterization of metabolites, structure elucidation, synthesis and experimental biosynthesis of natural Product as well as developments of methods in these areas. Research papers in the fields of chemistry-biology boundary, e.g. fermentation chemistry, plant tissue culture investigations etc. are also welcomed. Although the journal focuses mainly on original research articles, timely, concise and focused reviews on recent progress in active areas of natural Product is also encouraged.

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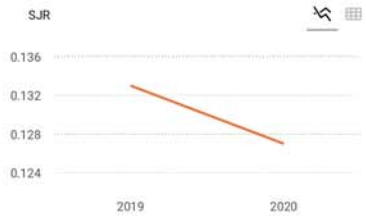

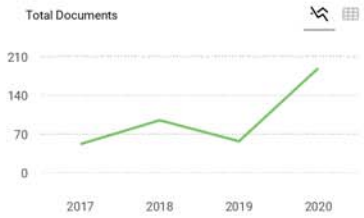

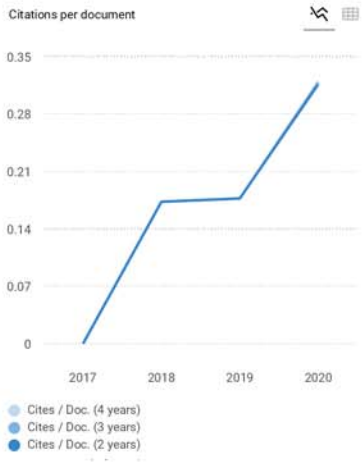

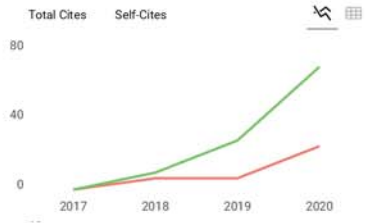

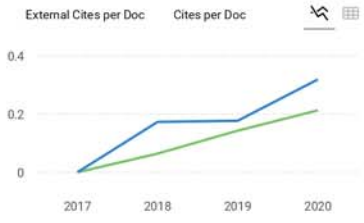

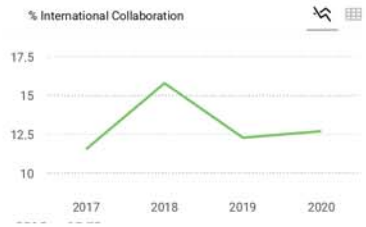

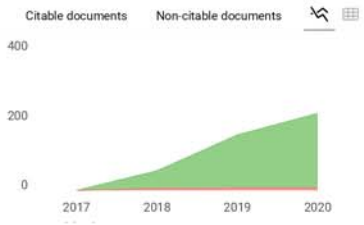

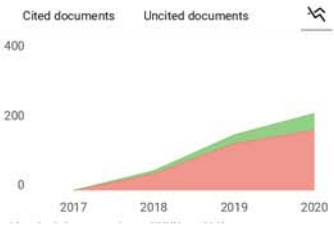

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Introduction
Cardiovascular compromise is common in sick term and preterm infants. Impaired myocardial contractility and low cardiac output are common complications of such conditions as respiratory distress syndrome and Perinatal Asphyxia (Clark et al., 2016).

This reduced cardiovascular reserve may present clinically with hypotension, which is associated with increased mortality and adverse neurological outcomes. It has been suggested that this myocardial dysfunction, or stunning, is due to ischemia and/or necrosis(So You et al., 2020).
Cardiac biomarkers are being increasingly incorporated into clinical trials as indicators of myocardial strain. Furthermore, they can possibly be used to guide therapy and improve outcome. They are potential tools in the diagnosis and treatment of neonatal disease that is complicated by circulatory compromise (Daniel et al., 2017).
Previous studies in neonates have used creatine kinase isoforms as Biochemical markers of myocardial injury. However, these markers have been largely discarded because gestation, sex, mode of delivery, and birth weight all affect creatine kinase activity (Clark et al., 2016)
Cardiac troponin T (cTnT) is a regulatory contractile protein whose detection in the circulation has been shown to be a specific and sensitive marker for ischemic myocardial cell injury both in adult and pediatric populations (Thygesenet al., 2017).
Specific forms of the three troponin subunits T, C, and I exist in different muscle types. Cardiac specific troponins T and I have become established as the best biochemical markers for myocardial necrosis (Nikhileshet al., 2015).
They start to increase two hours after myocardial infarction, and concentrations can remain raised for up to two weeks after a full thickness infarct (Nikhileshet al., 2015).
Cardiac troponin T is detectable in the blood of many healthy neonates, but no relation with important basic and clinical variables was found. Sick infants have significantly higher concentrations than healthy infants. The variations in cardiac troponin T concentration were significantly associated with oxygen requirement or the use of inotropic support in a regression model. Cardiac troponin T may be a useful marker of neonatal and cardiorespiratory morbidity (Clark et al., 2016)

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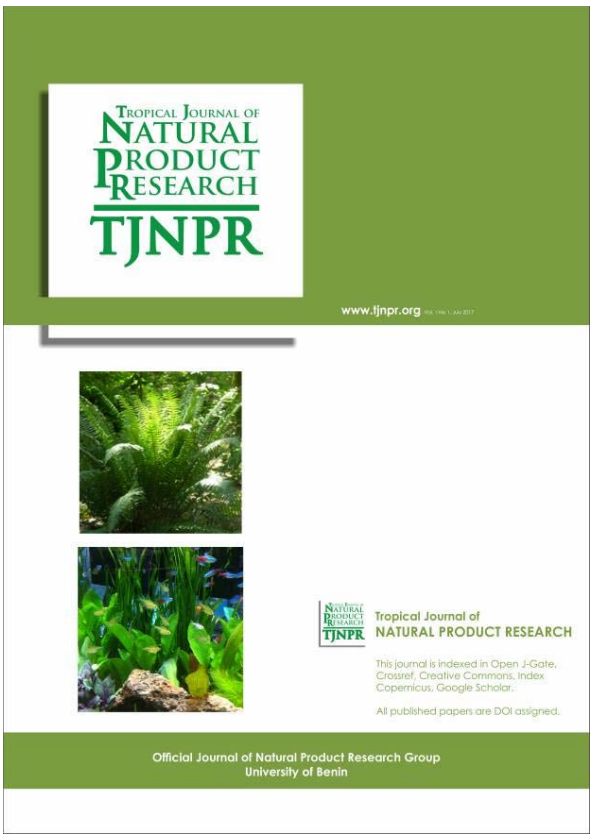

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