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Original Article

Antioxidant Capacity of Some Selected Medicinal Plants in East Nusa Tenggara, Indonesia: The Potential of *Sterculia quadrifida* R.Br.

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ABSTRACT

Background: Medicinal plants derived natural product such as alkaloids, flavonoids and terpenes. The traditional uses claim that medicinal plants of NTT are potential folk medicine but very little research has been conducted on these plants. **Objective:** The present study is directed towards evaluating naturally effective antioxidant of twenty-four traditional medicinal plants collected from Kupang, East Nusa Tenggara (NTT) using *in vitro* models. **Method:** The antioxidant activities were determined by using the free radical scavenging assays 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-2″-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Analysis of total flavonoid and phenolic contents were expressed as Quercetin Equivalent (QE) and Gallic Acid Equivalent (GAE), respectively. Statistical methods are used in data analysis include mean or standard deviation, and regression analysis. **Result:** Methanol extract of *Sterculia quadrifida* R. Br. root exhibited the highest DPPH radical scavenging activity with IC_{50} value of 3.11µg/mL, followed by *Schleichera oleosa* and *Euphorbia hirta* L. extracts with IC_{50} values of 10.05 and 10.09 µg/mL, respectively. Methanol extract *of S. quadrifida* also showed highest ABTS radical scavenging activity with IC_{50} value of 7.29 µg/mL, followed by *Eugenia jambolana* Lam. and *Lamea grandus* with IC_{50} value of 9.15 and 12.29 µg/mL, respectively. *S. quadrifida* extract showed high flavonoid and phenolic content with 661.85 mg of GAE and 116.84 mg of QE per 100 g of extracts. **Conclusion:** The present study gives scientific evidences that twenty four samples of NTT plants have high free radical scavenging capacity. Strong radical scavenging activity of these plants especially *S. quadrifida* could be considered as a potential source of natural antioxidants.

Key words: ABTS, Antioxidants, DPPH, Free radicals, Traditional medicines.

Key message: This study focus on *in vitro* antioxidant activity of twenty-four traditional medicinal plants, in order to explore the potential of medicinal plants of NTT.

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INTRODUCTION

Indonesia has abundant biodiversity which are thousands species of plants widely distributed in tropical rainforests including Timor island monsoon forest in East Nusa Tenggara (NTT) province. Dry soil structure and physicochemical properties with low rainfall intensities and water supplies led NTT to have a different biodiversity compare with another region of Indonesia.[1] Plants have various benefits of meeting the human demand of clothing, food, and shelter. Moreover, those plants are essential for health care as medicinal resources. Plants have been used as medicine since the ancestral period in curing diseases.[2-3] People in NTT only acquire their knowledge from their ancestors, without any scientific evidences before. Traditional medicinal plants are commonly used as an alternate treatment in order to cure the diseases until now. They still consume it because of their safety, effectiveness, and readily evaluable in rural area. This is because people are more concern about the side effects of several syntetic medicines which can be a toxic.[4-5]

The medicinal properties of traditional medicinal plants have been investigated in the recent scientific developments for treatment of many metabolic diseases. The extract of these plants from various places in the world are reported to possess several pharmacological activities including anti-inflammatory,[6-7] antidiabetic,[8-11] antimalarial,[12] antimicrobial,[13-14] anti TB,[15] anthelmintic activity,[16] anticancer,[17-18] cylindroxanthones A-C (1-3 etc. Traditional medicinal plants derived natural product such as alkaloids, flavonoids and terpenes. These compounds are the most important common sources of potentially natural antioxidant that can be used for prevention of degenerative diseases. Antioxidants are molecules which

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can prevent oxidation in other molecules through interact with free radical and terminate the chain reaction.[19] The primary characteristic of an antioxidant is its ability to trap free radicals. Free radicals are produced in human body as a by-products of body metabolism of an unhealthy life style. Free radicals do not only cause metabolism perturbation but also damage human tissue. Uncontrolled free radical leads to oxidative stress of tissue, ultimately cell death and subsequent diseases.[20-21]

The present study is directed towards discovering naturally effective antioxidant of plant origin from NTT. The traditional uses claim that medicinal plants of NTT are potential folk medicine but very little research has been conducted on these plants. The evaluation of twenty-four traditional medicinal plants endemic from NTT had been performed. The most active as antioxidant is *Sterculia quadrifida* R. Br. *S. quadrifida* is the most plant used in NTT traditional medicines. Traditionally, bark and roots extracts of *S. quadrifida* are commonly used to treat various diseases such as diabetic, liver and cancer. *S. quadrifida* locally known as Faloak is an endemic plant to NTT as shown in Figure 1. It grows wild throughout NTT including Timor, Sumba, Flores and Alor islands.[22] To the best of our knowledge, this is the first report on the antioxidant potency of crude extract from NTT traditional medicines including *S. quadrifida.*

The aim of this research is to find out total flavonoid content (TFC), total phenolic content (TPC), and *in vitro* antioxidant activity of extracted medicinal plants from NTT by using free radical scavenging assays DPPH and ABTS, in order to explore the potential of medicinal plants of NTT. This research would give scientific knowledge on NTT medicinal plants which are potentially used as antioxidant for further development of antioxidant.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-2”-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), trolox, quercetin, gallic acid and dimethyl sulfoxide (DMSO) were purchased from sigma chemical company (St. Louis, MO-USA), Folin-Ciocalteu reagent, Na_2CO_3, $NaNO_2$, $AlCl_3.6H_2O$, NaOH, ethanol 99.5 % and methanol were acquired from Merck (Darmstadt, Germany). Other chemicals used for the study were analytical grade.

Figure 1: *Sterculia quadrifida* R. Br. a). plant b). flower c). fruit d). seed

Table 1: Traditional uses of medicinal plants from East Nusa Tenggara (NTT).

Botanical Name of the Plant	Local Name of the Plant	Parts of Plant	Indications
Acalypha indica L.	Anting-anting	leaf	Dysentery, inflammation
Alstonia scholaris L.R.Br	Taduk	leaf	Malarial, dermatitis
Artemisia vulgaris L.	Baru cina	leaf	Dysentery, vomiting
Azadirachta indica A.	Nimba	leaf	Toothache, kidney
Bidens pilosa L.	Rumput kuda	leaf, bark	Diabetic, inflammation
Calotropis gigantean L.	Kolang susu	leaf	Dermatitis, wounds
Cassia siamea Lamk.	Johar	leaf	Diabetic, fever
Cassia tora L.	Pepo	leaf	Eye infections
Ceiba pentandra (L) Gaertn.	Kapuk	leaf	Cough, inflammation
Elephantopus scaber L.	Tapak liman	leaf	Inflammation, fever
Eugenia jambolana Lam.	Jamlang	leaf	Diabetes, cancer
Euphorbia hirta L.	Rumput Kerbau	leaf	Asthma, dysentery
Euphorbia tirucalli L.	Patah Tulang	leaf, bark	Rheumatism, dermatitis
Jatropha gossypifolia L.	Jarak merah	leaf	Inflammation, fever
Lamea grandus	Kedondong Hutan	leaf	Cough, dysentery
Mentha arvensis L.	Bunga putih	leaf	Wounds, toothache
Moringa oleifera Lam.	Kelor	leaf	Rheumatism, eye infections
Phyllanthus niruri L.	Cinta buah	leaf, bark	Kidney stones
Plectranthus amboinicus Lour (L) Spreng.	Retunu Rote	Leaf	Cough, fever, asthma
Sauropus giganteus (L.) Merr.	Katuk	leaf	Cholesterol, diabetic
Schleichera oleosa	Kesambi	leaf	Eczema, dermatitis
Solanum tarvum	Terong hutan	fruit	Cough, boil
Sterculia quadrifida R. Br.	Faloak	root	Diabetes, cancer
Strychnos lucida R. Br.	Kayu Ular	leaf	Malarial, diabetic

Plant materials

Plants were collected from some areas around Kupang district, NTT province, Indonesia. The samples used were leaves, roots, and barks of the medicinal plants were shown in Table 1. Voucher specimens were identified at Purwodadi Botanical Garden, East Java. Specimens of the collected plants were deposited in the laboratory of Natural Product Chemistry and Synthesis, department of Chemistry, Institut Teknologi Sepuluh Nopember, Surabaya.

Extraction

The plant sample was air-dried to eliminate the humidity. Then, it was pounded into a fine powder. Next, it was weighed for 100 g and was extracted by maceration with 400 mL methanol in 24 h at room temperature. Then the extract was filtered and evaporated under vacuum by using *rotary evaporator* to dryness.

Antioxidant assay

Producing DPPH dissolvent

The molecule formula of DPPH is $C_{18}H_{12}N_5O_6$ with molecular weight 394.32 g/mol. DPPH dissolvent is 6 x 10^{-5}M made by dissolving 0.24 mg of DPPH in 10 mL methanol.

DPPH radical scavenging assay

DPPH assay was determined by the method of Brand Williams modified by Dudonn'e *et al.*[23-24] About 3 mL of 6 x 10^{-5} M DPPH solution was mixed with 100 µL samples in a test tube. After being incubated for 20 min at 37°C of temperature, absorbance decrease of the mixture was determined at 515 nm in a UV-Visible Spectrophotometer (*As*). Radicals DPPH have maximum absorbance at about 515 nm (*Ab*). The absorbance of *blank* of DPPH solution was also measured at the same wavelength (*Ab*). Trolox was used as positive control. The experiment was performed in triplicate. Radical scavenging activity was calculated by using the following way.

$$\text{Inhibition rate}\,(\%) = \left[\frac{A_b - A_s}{A_b}\right] \times 100$$

ABTS radical scavenging assay

ABTS radical scavenging assay were described by Re *et al.*[25] About 5 mL of 7 mM ABTS ammonium aqueous solution was mixed with 88 µL of potassium peroxydisulfate ($K_2S_2O_6$) 140 mM. The mixture was allowed to stand for 12-16 h at room temperature to yield a dark blue solution. Then it was mixed with 99.5 % of ethanol which gave 0.7 ± 0.02 units absorbance at 734 nm to get working solution. One mL of working solution was mixed with 10 µL of samples extract and shuffled for 10 sec, then incubated at 30°C of temperature for 4 min. Then, the absorbance of the mixture reaction was measured at 734 nm to produce *As* values. Ethanol 99.5% was used as a *blank* solution and its absorbance was measured to produce *Ab* value. Trolox was used as positive control. The inhibition rate was measured by the same formula as shown in DPPH assay.

Estimation of Total Flavonoid Content (TFC)

The TFC was determined using the Aluminum chloride colorimetric assay described by Zhishen *et al.*(1999)[26] with some minor modifications. The extract was dissolved in methanol at a concentration of 100 µg/mL and distilled water was added to make 5 mL. A 0.3 mL 5% $NaNO_2$ was added to the flask. After standing for 5 min at room temperature, 0.3 mL 10% $AlCl_3.6H_2O$ was added to the flask and was allowed to stand for 6 min at room temperature. 2 mL 1 M NaOH was then added and the total volume was made up to 10 mL with distilled water. After a thorough mixing, the absorbance reading was recorded at 510 nm using a UV-visible spectrophotometer. Results were expressed as mg quercetin equivalents (QE)/100g of extract. All samples were analyzed in triplicate.

Estimation of Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu (FC reagent) assay following Singh *et al.* (2013).[27] The extract was dissolved in methanol at a concentration of 100 µg/mL. Then, these sample solutions were mixed with 3.6 mL of water and 0.4 mL of FC reagent 10% (v/v) and were allowed to stand for 5 min at room temperature. 4 mL 7% Na_2CO_3 solution was then added. The solutions were made up to 10 mL with water mixed. After 90 min, the absorbance of each samples was recorded at 760 nm using a UV-visible spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE)/100g of extract. All samples were analyzed in triplicate.

RESULTS

Antioxidant activity of traditional medicinal plants from NTT has been determined by using two measuring methods, DPPH and ABTS free radical scavenging assays. All the medicinal plants showed varying free radical scavenging activities as potent antioxidant when compared to trolox as a standard. There was a wide range of antioxidant capacities of medicinal plant extracts using DPPH assay. The DPPH inhibition rate varied from 0.8 to 90.06 %. The reduction capacity of DPPH radical shown by absorbance decrease at 515 nm induced by antioxidants. *S. quadrifida* was reported as a strong potential antioxidant plant with 80.13 % of radical scavenging capacity. Other antioxidant compound resource exists in *Euphorbia hirta* L. and *Lamea grandu* with 90.06 and 89.92 % of DPPH inhibition rate successfully. Trolox was taken as standard showed 97.89 % antioxidant activity. Whereas *Solanum tarvum*, *Euphorbia tirucalli* L. and *Alstonia scholaris* L.R. Br showed low level of antioxidant capacity with 0.80; 3.79 and 6.85 % of DPPH inhibition rate respectively from selected plants. The result of DPPH radical scavenging at 319.45 ppm of medicinal plant extracts is shown in Figure 2.

To confirm the antioxidant activities using DPPH radicals, we examined the IC_{50} values of six plant samples which had the highest activity in the screening result (Table 2).

The experimental results showed the highest antioxidant activity by DPPH method was observed in *S. quadrifida* plant extract with IC_{50} value of 3.11 µg/mL. The low IC_{50} value indicate high antioxidant activity of

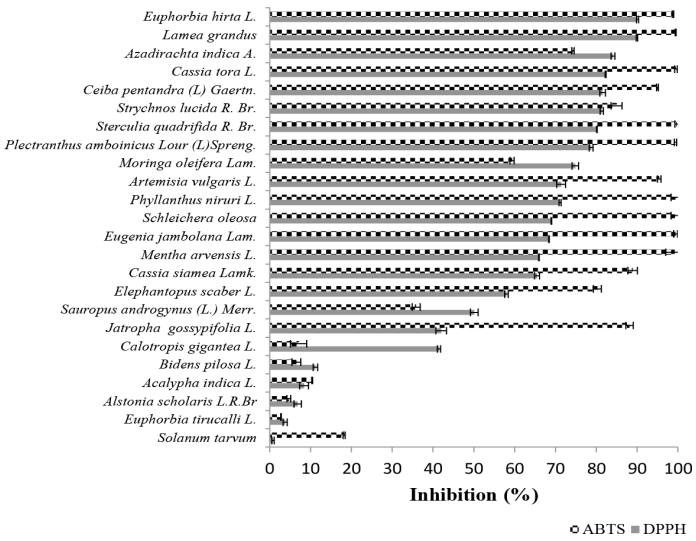

Figure 2: Free radical scavenging activity of 24 NTT medicinal plant extracts at a concentration of 319.45 µg/mL for DPPH and 99 µg/mL for ABTS; triplicate experiments.

Table 2: Antioxidant activity of potent NTT traditional medicinal plants using DPPH assay.

Botanical Name	Local Name	IC_{50} (µg/mL)
Sterculia quadrifida R. Br.	Faloak	3.11
Schleichera oleosa	Kesambi	10.05
Euphorbia hirta L.	Rumput Kerbau	10.09
Eugenia jambolana Lam.	Jamblang	16.22
Lamea grandus	Kedondong Hutan	27.80
Phyllanthus niruri L.	Cinta buah	31.63

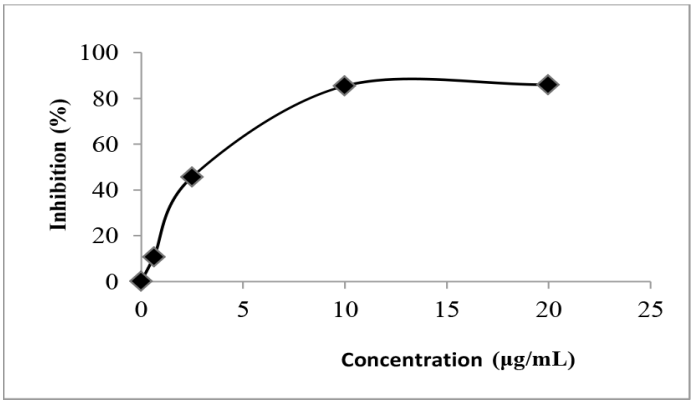

Figure 3: DPPH radical scavenging activity of *Sterculia quadrifida* R. Br.

Table 3: Antioxidant activity of potent NTT traditional medicinal plants using ABTS assay.

Botanical Name	Local Name	IC_{50} (µg/mL)
Sterculia quadrifida R. Br.	Faloak	7.29
Eugenia jambolana Lam.	Jamblang	9.15
Lamea grandus	Kedondong Hutan	12.29
Schleichera oleosa	Kesambi	18.27
Euphorbia hirta L.	Rumput Kerbau	18.35
Phyllanthus niruri L.	Cinta buah	37.39

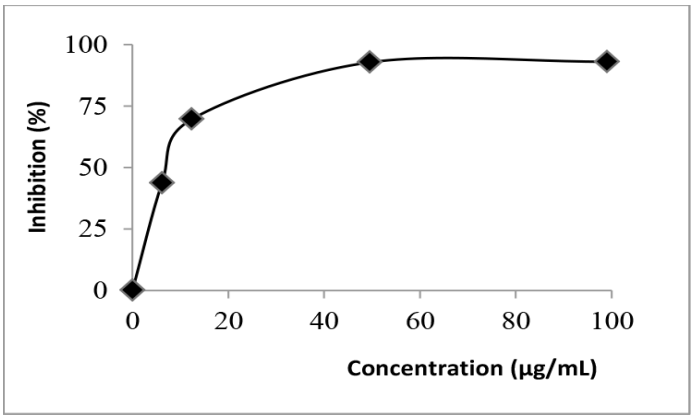

Figure 4: ABTS radical scavenging activity of *Sterculia quadrifida* R. Br.

the substrate. Figure 3 shows inhibition rate of *S. quadrifida* using DPPH radical scavenging assay.

The free radical scavenging has also determined using the ABTS scavenging assay. ABTS radical scavenging activities of medicinal plants varied from 2.78 to 99.66 %. The reduction of radical ABTS is shown by the decreasing of absorbance at 734 nm. The highest antioxidant capacity was observed in extract of *S. quadrifida* with 99.66 % of ABTS inhibition rate, followed by *Cassia tora* L. and *E. jambolana* with 99.49 and 99.42 % respectively. Whereas *E. tirucalli*, *A. scholaris,* and *Bidens pilosa* L. showed low antioxidant capacities values of 2.78; 4.69 and 6.50 % of inhibition rate successfully. Trolox was taken as standard showed 95.97 % antioxidant activity. The result of ABTS radical scavenging at 99 ppm of medicinal plant extracts is shown in Figure 2. Table 3 showed the IC_{50} value of NTT traditional medicinal plants from ABTS assay.

In this study, the highest antioxidant activity by using ABTS is found in *S. quadrifida* with IC_{50} value of 7.29 µg/mL. The extracts of *E. jambolana* also have strong antioxidant capacity with IC_{50} values of 9.15 µg/mL followed by *L. grandus* with IC_{50} values of 12.29 µg/mL. ABTS radical scavenging activity of *S. quadrifida* is shown in Figure 4.

Table 4: Total flavonoid and phenolic content of potent NTT traditional medicinal plants.

Botanical Name	Local Name	Total Flavonoid Content (mg of QE/100g of extract)	Total Phenolic Content (mg of GAE/100g of extract)
Sterculia quadrifida R. Br.	Faloak	661.85 ± 12.83	116.84 ± 1.22
Schleichera oleosa	Kesambi	595.19 ± 6.42	176.84 ± 1.05
Phyllanthus niruri L.	Cinta buah	398.89 ± 11.11	9.47 ± 1.05
Eugenia jambolana Lam.	Jamblang	180.37 ± 12.83	62.11 ± 1.61
Euphorbia hirta L.	Rumput Kerbau	84.07 ± 6.42	75.79 ± 1.22
Lamea grandus	Kedondong Hutan	24.81 ± 6.42	20.0 ± 1.61

An extensive range of flavonoid and phenolic content were estimated in medicinal plant extracts. The highest total flavonoid content was observed in extract of *S. quadrifida* with 661.85 mg of quercetin equivalents (QE) per 100 g of extracts, followed by *S. oleosa* with 595.19 mg of QE per 100 g of extract. Quercetin is used as a standard for flavonoid content. *S. quadrifida* also showed a high total phenolic with 116.84 mg of gallic acid equivalents (GAE) per 100 g of extract as shown in Table 4. Gallic acid is used as a standard for phenolic content. In TPC test, it is considered that a complex is formed between extract and reagent indicated by colour changes from yellow to blue.

DISCUSSION

In the present study, twenty four traditional medicinal plant extracts from NTT were identified to have antioxidant activity. The experiments result showed there were six most active extracts among others, *S. quadrifida, S. oleosa, P. niruri, E. jambolana, E. hirta,* and *L. grandus.* It revealed the free radical scavenging capacities of the selected medicinal plant extracts using DPPH and ABTS assay. DPPH and ABTS radical scavenging are based on electron transfer, in which those methods measure the capacity of an antioxidant to reduce an oxidant, which changes colour when reduced.[26-27]

DPPH is a stable free radical which will accepts electron or hydrogen radical to form a more stable molecule. Interacted with DPPH, antioxidant will transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical and convert it to 1, 1-diphenyl-2-picrylhydrazine. The determination of free radical scavenging activity by DPPH method was influenced by some important factors such as dissolving liquid, the length of reaction, the ratio of sample and reagent, and the wavelength for measuring the absorbance. Antioxidant capacities of extracts not only depend on extract composition, but also on the conditions of the test used.[28-29] The reduction of the DPPH molecules by the molecules of the substrate was visually shown by a change in colour of purple DPPH solution turned to light yellow when mixed with plant extract by 20 min incubation period. This process shows that antioxidant compound reacted with radical DPPH for it was reduced to DPPH-H which is more stable and signed by the reduction of absorbance value of DPPH. The reduction degree of absorbance indicated the radical scavenging ability of the extract. Plants have high inhibition rate which shows them as strong antioxidant and vice versa.[30]

The phenolic and flavonoid content in plant extracts impacts significantly to their antioxidant potential which might be responsible for radical scavenging activity. Various study revealed that phenolic and flavonoid are the most important substance that present numerous biological activity and pharmacology properties.[31] The results of this study support that medicinal plants in NTT is rich in phenolic and flavonoids that might contribute to potential antioxidant activities.

S. quadrifida is a well-known as NTT traditional medicinal plants. Traditionally bark and roots extracts of *S. quadrifida* have been proved to be useful for the treatment of diabetic, liver and cancer. In this work, *S. quadrifida* showed a highest antioxidant activity with DPPH and ABTS assays, and it also have a high total flavonoid and phenolic content. Free radical can diffuse throughout the body and attack important biomolecules including DNA leading to oxidative damage of DNA. The resulting damage is involved in mutagenesis, carcinogenesis and aging. Phytochemicals including flavonoid and phenolic can prevent damage by their radical scavenging ability.[32]

The secondary metabolites found in one species in a genus will be similar to another species in the same genus. Flavonoid, terpenoid, alkaloid, phenolic and steroid were reported to exist in other species of genus *Sterculia,* but there is no literature reports found on the chemistry of *S. quadrifida*. It offers a challenge in finding new constituent from *S. quadrifida*. Study of genus *Sterculia* plant extracts revealed some biological activity like anti-diabetic from *S. villosa*,[5] anti-inflammatory and antifertility activity from *S. foetida,*[33] larvacidal activity from *S. guttata,*[34] and anti-proliferative activity from *S. tavia.*[35] Some chemical constituents also reported from genus *Sterculia* plants such as sitosterol and betulinic acid were isolate from *S. striata,*[36] sterculinine I and II were isolate from *S. lychnophora,*[37] and 1,6-diferuloyl glucose was isolate from *S. foetida.*[38] Although the genus *Sterculia* has been well investigated, *S. quadrifida* has not been explored for its chemical constituents and biological activity. Further investigation on isolation and identification of bioactive constituents derived from *S. quadrifida* is in progress.

CONCLUSION

The present study gives scientific evidences that twenty four samples of NTT plants have high free radical scavenging capacity. *S. quadrifida* is a promising candidate for antioxidant investigation for future studies.

Methanol extract of *S. quadrifida* root exhibited the highest DPPH and ABTS radical scavenging activity with IC_{50} value of 3.11 and 7.29 µg/mL, respectively. *S. quadrifida* extract also showed high flavonoid and phenolic content with 661.85 mg of GAE and 116.84 mg of QE per 100 g of extracts. The result is a valuable reference of antioxidant properties from NTT traditional medicinal plants, which is serve as scientific value of antioxidant source in Indonesia.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ABBREVIATIONS

NTT: Nusa Tenggara Timur, **DPPH:** 1,1-diphenyl-2-picrylhydrazyl, **ABTS:** 2-2”-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), **QE:** Quercetin Equivalent, **GAE:** Gallic Acid Equivalent, **TFC:** Total Flavonoid Content, **TPC:** Total Phenolic Content, **DIKTI:** Direktorat Jenderal Pendidikan Tinggi, **TYKL:** Theodore Yehezkiel Kristoferson Lulan, , **TB:** Tubercle bacillus, **FC:** Folin-Ciocalteu, **DMSO:** dimethyl sulfoxide.

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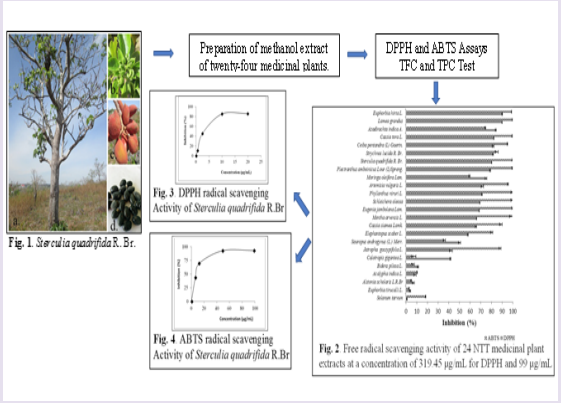
GRAPHICAL ABSTRACT



SUMMARY

- Twenty-four traditional medicinal plants collected from Kupang, East Nusa Tenggara (NTT) were determined for antioxidant activity by using the free radical scavenging assays DPPH and ABTS. The present study revealed that the methanol extract of *Sterculia quadrifida* R. Br. root exhibited the highest DPPH and ABTS radical scavenging activity. It also showed high flavonoid and phenolic content. Strong radical scavenging activity of these plants especially S. quadrifida could be considered as a potential source of natural antioxidants.

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