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Comparative study on free radical ameliorating potential of stem and the leaf extracts of *Plectranthus amboinicus*

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The *Plectranthus amboinicus* is known for its medicinal properties. The extracts from this plant material are used to treat different diseases which include cold, respiratory disorders, digestive problems, skin infections, cough, chronic asthma, bronchitis, hiccups, etc. The present study aimed to compare and evaluate the antioxidant property of two aerial parts of the plant, the stem and the leaf extracts of *Plectranthus amboinicus*. The different type of extracts was prepared with fresh leaves, fresh stem and dried leaves, with methanol as the solvent for extraction. The antioxidant potential of each extract was checked and the result obtained showed the fresh stem extract had the highest antioxidant activity. The study result shows that the methanolic extracts of dried leaves have Tannins, flavonoids, phenol and reducing sugars. The methanolic extract of fresh leaves and fresh stem extracts showed the presence of Tannins, flavonoids, quinone, steroids, phenols and reducing sugars. The phenolic content of the methanolic extract of the stem is high compared to fresh and dried leaves. The anti-oxidant potentials were assayed through the FRAP, FTC and TBA assays, the results showed that the free radical scavenging property of the methanolic extract of the stem is high compared to the fresh and dried leaves. The methanolic stem extract has high phenolic content and also shows high free radical scavenging properties than the methanolic extract of fresh and dried leaves.

Key words: Plectranthus amboinicus, FRAP, FTC, anti-oxidant activity, phenolic content, phytochemicals

Ancient traditional medicines were the base of all knowledge, skills, and practices of various cultures to identify and treat mental and physical illnesses. Herbal medicines are commonly used by people due to their medicinal value and therapeutic effect. The use of medicinal plants to cure diseases has become regular in most populations (Achour *et al*., 2022). Herbal medicine and its importance in healthcare have attracted attention among botanists to have a better understanding of the traditional knowledge, ecology and diversity of herbal and Phytomedicines. WHO expert groups have also emphasized the importance of medicinal plants in the diagnosis and treatment of various fatal diseases. Moreover many medicinal plants are consumed locally as drinks, herbs and foods. The extracts of the herb were used as an ointment to treat dermatitis, abrasion and wound. Inhalations of odour from the medicinal plants to treat chest and nasal infections are also common (Mengistu *et al*., 2022). Developing countries have used medicinal plants numerous times for treating diseases. Because of their easy availability with no side effects when compared to modern medicines, medicinal plants have played a pivotal role in India. There are many ethnomedical plants in the champhai district that lies in the indo-Burma regions (Laldingliani *et al*., 2022).

There is a huge demand for medicinal plants in developing countries putting high pressure on the plant populations. Many medicinal plants are cultivated from wild populations for informal trade but this cultivation is not regulated (Greenwell *et al*., 2016). Herbal medicines have played a crucial role in health systems and are used to treat different chronic and acute diseases with minimal or nil toxic effects. Diseases like tuberculosis, diabetes mellitus, cancer, and heart diseases are often treated with a natural remedy that contains the medicinal properties of herbal plants. India which is a rich depository of medicinal plants and other herbal practices is considered a “living tradition” in the use of medicinal plants. But there is no accumulative report on the significant medicinal plants and their progress in research (Prasath Kumar *et al*., 2021).

Fig. 1 *Plectranthus amboinicus*

SYSTEMIC CLASSIFICATION

Kingdom: *Plantae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Plectranthus*

Species: *P. amboinicus*

Morphological Features

Plectranthus amboinicus is a succulent shrub with a tendency for climbing or creeping. It can reach over 1 m in height and even more in width in the wild. This sprawling large succulent herb is fleshy and highly aromatic. The fleshy stems grow about 30–90 cm, either with long rigid hairs (hispid villous), or tomentose (densely covered with soft, short and erect hairs, pubescent). Leaves are undivided (simple), broadly ovate to suborbicular with a tapering tip (ovate) and very thick; they are pubescent (thickly studded with hairs), with the lower surface possessing the most numerous glandular hairs, giving a frosted appearance. The taste of this leaf is pleasantly aromatic with an agreeable and refreshing odor. Flowers are on a short stem (shortly pedicelled), pale purplish in dense whorls at distant intervals in a long slender raceme. Flowers have a bell-shaped calyx and the throat is smooth inside with two lips, the upper lip being ovate and thin, the lower lip having four narrow teeth. The corolla is pale purplish

and five times longer than the calyx, with a short tube, inflated throat and short lips. Fruit nutlets are smooth, pale brown in color, 0.7 mm long and 0.5 mm wide. *P. amboinicus* rarely flowers and seeds are difficult to collect. (Greetha Arumugam *et al.,* 2016).

Belonging to the family Lamiaceae, *Plectranthus amboinicus* is commonly called parnayavani. The plant is well known for its medicinal property to treat common cold, cough, abdominal flatulence constipation and flatulence (Sahu *et al*., 2022). This plant is distributed in the African tropical and warm regions and also in Australia and Asia. The herb has nutritional and therapeutic attributes with its phytochemical compounds that have apex values in pharmaceutical industries. A literature survey has revealed that 76 volatiles and 30 non-volatile compounds of *P. ambonicus* belong to phytochemicals including sesquiterpenoids, phenolics, flavonoids, aldehydes and esters. Numerous studies have reported with antimicrobial, antitumor, anti-inflammatory, wound healing and analgesic activities of the plant. Studies also reveal *P. Amboniicus* is effective against cardiovascular, oral, digestive, respiratory and urine diseases (Arumugam *et al*., 2016). The ethanolic extracts of *Plectranthus omatus* contain tannins, saponins and flavonoids. It also contains anthocyanins and anthocyanidins. The flavonoids content of the plant is composed of cirsimartin, rutin, salvigenin, quercetin and apigenin (Rodrigues *et al*., 2021). Antioxidant activity of P. omatus extracts, isolated compounds and fractions were estimated using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay that measures the scavenging radical activity of the samples. Ascorbic acid is used as a standard (Mothana *et al*., 2019). The present study was to compare the free radical ameliorating potential of stem and the leaf extracts of *Plectranthus amboinicus.*

MATERIALS AND METHODS

Plant material Collection

Fresh leaves (1200 gm) and stems (25 gm) of the selected plant (*Plecranthus amboinicus)* were collected from Kristu Jayanti College, K. Narayanpura, Bengaluru. The collected plant material (leaves) were washed, cleaned and dried at 50^0 C in a hot air oven until they attained a constant weight. The samples were then crushed into powder, using a mechanical grinding machine, so as to enhance the effective contact of the solvent with the plant materials.

Crude extraction

Three different extracts were used in this experiment- the methanol which has higher elemental oxygen content, a lower heating value and also has shown high activity in the preliminary analysis for this plant.

1. Dried leaf extract: The leaves were collected and washed under running tap water. Leaves were kept for drying in a hot air oven at 50^0 C. The dried leaves were powdered and 25gm of powdered leaves were subjected to soxhlet with 250ml of methanol used as the solvent. After seven cycles of soxhlet extraction, the solvent was removed from the sample by evaporating at 50^0 C using a water bath till the sample get dried. The residue was dissolved in 50ml of methanol and used for the study (Pillai *et al.,* 2013).

2. Fresh leaf extract: Fresh plant material (leaves) was washed and cut into small pieces. 25 gm of measured plant material was soaked in 250ml of methanol and kept at room temperature for 24 hrs. Filtered the solution and the filtrate was evaporated to dryness at 50^0 C using a water bath. The residue was dissolved in 50ml of methanol and used for the study (Nair *et al*., 2016).

3. Fresh stem extract: Fresh plant material (stem) was washed and cut into small pieces. 25 gm of measured plant material was soaked in 250ml of methanol at room temperature for 24 hrs. Filtered the solution and the filtrate was evaporated to dryness at 50 c using a water bath. The residue was dissolved in 50ml of methanol and used for the study (Maya S. Nair *et al*., 2016).

Phytochemical Screening

Extracts were subjected to qualitative analysis to test the various phytochemical components present in the samples using the method developed by (Soni and Sosa., 2013). The different tests performed are as follows:

1 Test for Tannins

0.5ml of each extract was taken in different test

tubes and 1ml of potassium ferricyanide (0.008M) was added to each tube.1ml of ferric chloride (0.02M) containing 0.1N HCL was added and observed for blue-black coloration.

2 Test for Flavonoids

0.5 ml of each extract was taken in different test tubes and 5ml of dilute ammonia solution was added to each tube followed by the addition of concentrated H_2SO_4. A yellow coloration indicates the presence of flavonoids.

3 Test for Quinones

0.5 ml of each extract was taken in different test tubes. 1 ml of NaOH was added to each tube. Blue-green or red coloration indicates the presence of quinines.

4 Test for Anthraquinones

0.5 ml of each extract was taken in different test tubes and boiled with 10 ml of H_2SO_4. 5 ml of chloroform was added to each tube and shaken well. The chloroform layer was pipetted into another tube and diluted with 1ml of 10% ammonia solution. The resulting solution was observed for colour changes as an indication of the presence of Anthraquinones.

5 Test for Steroids

0.5 ml of each extract was taken in different test tubes and 2ml of acetic anhydride was added to each tube. To this, 2ml of concentrated sulphuric acid was added. The colour changed from violet to blue or green indicates the presence of steroids.

6 Test for Phenols

0.5 ml of each extract was taken in different tubes and 1ml of NaOH was added. To this, 1ml of phosphomolybdic acid and 1ml of FC reagent were added. The formation of bluish-green colour indicates the presence of phenols.

7 Test for reducing sugar

To 0.5ml of each extract in a test tube, Fehling's Solution A and B were added and then kept in a boiling water bath. The reddish brown coloration indicates the presence of reducing sugars.

Evaluation of Antioxidant activity

The antioxidant activity of the *Plectranthus amboinicus* extracts was tested using three methods: Ferric reducing antioxidant power (FRAP), Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of the lipid peroxidation, in which peroxide reacts with ferrous chloride and forms a ferric ion. The ferric ion then combines with ammonium thiocyanate and produces ferric thiocyanate. The substance is red in colour. The higher the intensity of the colour, the higher will be the absorbance. Whereas the TBA method measures free radicals present after peroxide oxidation.

FRAP Assay

0.2 -1 ml of the standard was pipetted out into clean dry test tubes. 0.5ml of each extract was added to test tubes and labeled as a test (T1-T4). Then3.8 ml of FRAP reagent [200 ml of 300mM acetate buffer pH 3.6,20ml of 40mM of 2,4,6-tripyridyl-s-triazine(TPTZ) solution, 20ml of 20Mm of FeCl3.6H2O, 1.46ml of 40mM of HCl and 24ml of distilled water] was added in all the tubes. The above reaction mixture was incubated for 30 minutes at 37c. After incubation; the absorbance was measured at 600 nm against a blank using ascorbic acid (100 mg in 100 ml) as standard.

Ferric thiocyanate (FTC) method

The standard method as described by (Kikuzaki *et al*., 1993) was used.1ml of each plant extract was taken in a different vial. 4ml of absolute ethanol, 4.1ml of 2.5% linolenic acid in absolute ethanol, 8.0ml of 0.05M phosphate buffer (pH 7.0) and 3.9ml of water were placed in each vial with a screw cap and then placed in an oven at 40c in the dark. To 1ml of this solution was added 9.7ml of 7.5% ethanol and 0.1ml of 30% ammonium thiocyanate. Precisely 3 minutes after the addition of 0.1 ml of 0.02M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red colour was measured at 600nm every 24 hours until the day after absorbance of control reached the maximum. BHT (Butylated hydroxytoluene) and α-tocopherol were used as positive control while the mixture without plant sample was used as the negative control. (Maryam *et al*., 2015)

Thiobarbituric acid (TBA) method

The method of Ottolenghi, in 1959 was used to determine the scavenging activity. Two ml of 20% trichloroacetic acid and 2ml of 0.67% 2-thiobarbituric acid were added to 1ml of sample solution, as prepared in the FTC method. The mixture was placed in a boiling water bath for 30 minutes and after cooling was centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 600nm. Antioxidant activity was based on the absorbance of the final day of the FTC method. (Maryam Zahin *et al.,* 2009). The free radicals scavenging activity of the extracts were calculated using the formula.

$$\text{Scavenging activity \%} = \frac{(\text{A control} - \text{A sample}) \times 100}{\text{A control}}$$

Determination of Total phenolic content (TPC)

0.2ml-1ml of the standard (resorcinol) was pipetted out into clean dry test tubes. 0.5 ml of each extract was added to test tubes labeled as Test. Followed by 4.5ml of water and 1ml of FC reagent (Folin Ciocalteu) were added to each tube. All the tubes were incubated for 5 minutes. Then 5ml of 7% Na2 was added to each tube and incubated for 90 minutes. After incubation, the absorbance was measured at 600 nm against a blank. (AnjaliSoni and Sheetal Sosa, 2013)

Statistical Analysis:

Tests were carried out in triplicates. The results are expressed as mean ± S.E.M. Statistical analysis was performed using Student's t-test. P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Qualitative phytochemical studies were performed to screen the presence of different phytoconstituents in different extracts. The qualitative analysis identified the presence of six different phytochemicals which include Tannins, Flavonoids, Quinones, Steroids, Phenols, and Reducing sugars. The phytochemical constituents present in different extracts were shown in table 1.

Antioxidant activity

The results shown in table 2 indicates that the extracts have free radical scavenging property through FRAP, FTC and TBA assay of the methanolic extracts (dried leaf, fresh leaf and fresh stem) of *Plectranthus amboinicus.* The fresh leaf extract has the highest percentage of scavenging activity compared to the other two extracts with a statistical significance of 5%. Thus the extracts possess significant antioxidant activity.

Total phenol content

Table 3 represents the total phenolic content of all the three extracts (Dried leaves, fresh leaves and fresh stem) which varied significantly. The highest phenolic content was present in fresh stem extract.

The TPC was found to be higher in methanolic extract of fresh leaves and fresh stem of *Plectranthus amboinicus* than the methanolic extract of dried leaves and aqueous extract of fresh leaves of *Plectranthus amboinicus.*

The phenolic content of the stem methanolic extract of *Plectranthus amboinicus* is high than the fresh and dried leaves. Comparative study of the scavenging property through FRAP, FTC and TBA assays of methanolic extract of fresh leaves, dried leaves and fresh stem of *Plectranthus amboinicus* shows the scavenging property is more in methanolic extract of the stem than in fresh and dried leaves. The fresh leaves' methanolic extract has more scavenging ability than the dried leaves. This shows the stem has better activity than fresh and dried leaves.

Phenolic compounds play a pivotal role in the scavenging efficiency of plant extracts. The higher the phenolic content the higher will the scavenging property (Nguyen *et al.,* 2020). It is reported in general the methanolic or aqueous extraction of phenolic compounds is highest (Negi *et al*., 2005). The *Plectranthus amboinicus* plant extract has a good amount of phenolic content.

The unique structure of Phenolic compounds present in plant extracts contributes to their antioxidant potential. Phenolic compounds possess one (or more) aromatic rings with single or multiple hydroxyl groups. This structure contributes to the potential quenching of free radicals by forming resonance-stabilized phenoxyl radicals (Bors *et al*., 2002).

Plectranthus amboinicus essential oil has been widely used in folk medicine for the treatment of anxiety, memory deficit, and cancer due to its high antioxidant activity and antibacterial properties. Anti-oxidant

capability plays a significant role in the pathogenesis of a number of diseases and disorders such as inflammation, rheumatoid arthritis, asthma, psoriasis and contact dermatitis leading to oxidative stress, Free radicals can also cause damage to DNA which leads to mutagenicity and cytotoxicity. Thus it plays pivotal role in carcinogenesis. These non-toxic antioxidant complexes of transition metals with Flavonoids could be of importance for the suppression of toxic effects of iron and copper ions released from iron- or copper-containing proteins under certain pathological conditions (Ramani *et al*., 2021).

In summary, the present study showed the presence of six biologically active constituents from *P. ambonicus* including Tannins, Flavonoids, Quinones, Steroids, Phenols, and Reducing sugars. The anti-oxidant potential of the different extracts of *P. ambonicus* was studied using FRAP, FTC, and TBA along with the total phenol content. The present study showed high phenolic content with a wide scavenging efficacy.

Table 1. Phytochemical Constituents of *Plectranthus amboinicus* extracts

Phytochemical tests	Dried leaves (Methanol)	Fresh leaves (Methanol)	Fresh stem (Methanol)
Tannins	+	+	+
Flavonoids	+	+	+
Quinons	_	+	+
Anthraquinones	_	_	_
Steroids	_	+	+
Phenols	+	+	+
Reducing sugars	+	+	+

*(+) indicates presence of the Phytochemical constituent.
*(-) indicates absence of the Phytochemical constituent.

Table 2. Anti-oxidant assay of *Plectranthus amboinicus* extracts

S. No.	Sample	Solvent	Scavenging activity [%]		
			FRAP	FTC	TBA
1	Dried leaf	Methanol	39 ±0.103	29.50 ±0.108	16.66 ± 0.125
2	Fresh leaf	Methanol	69.23 ±0.023	55.73 ±0.047	75 ±0.053
3	Fresh stem	Methanol	48.71 ±0.043	34.42 ±0.045	25 ± 0.047

Each value represents the mean ± SEM; n=3; $p<0.05$.

Table 3: Total Phenol Content of the Methanolic extracts of *Plectranthus amboinicus*

S. No	Solvent	Test Samples	Total Phenolic content (µg)
1	Methanol	Dried leaves	165 ± 0.070
2	Methanol	Fresh leaves	175 ± 0.064
3	Methanol	Fresh stem	295 ±0.064

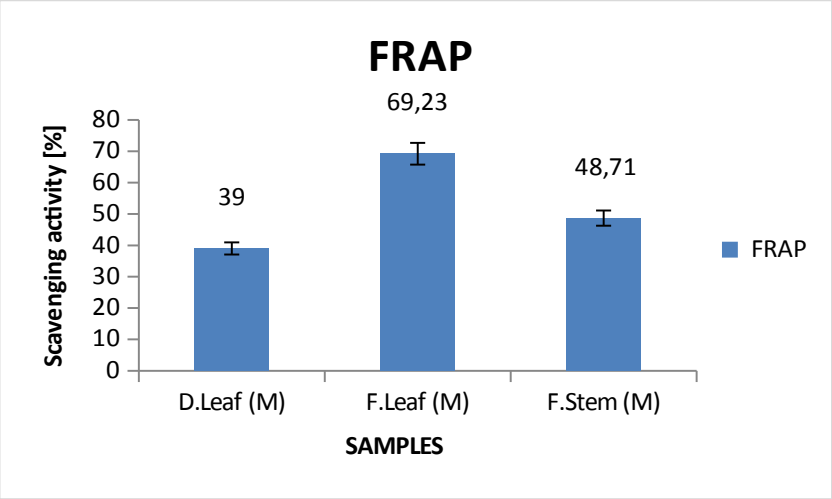

Figure 2 Graphs represents the percentage of scavenging activity [FRAP] of *Plectranthus amboinicus* extracts. Each value represents the mean ± SEM; n=3; p<0.05.

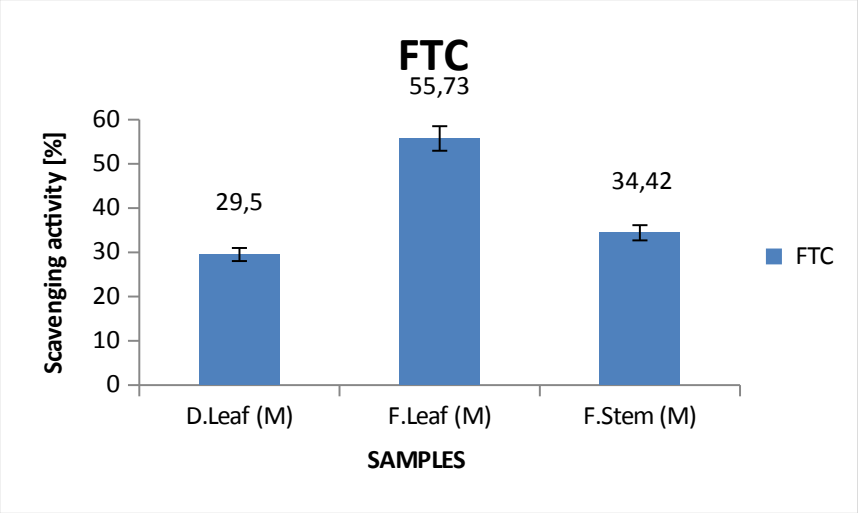

Figure 3 Graphs represents the percentage of scavenging activity [FTC] of *Plectranthus amboinicus* extracts. Each value represents the mean ± SEM; n=3; p<0.05.

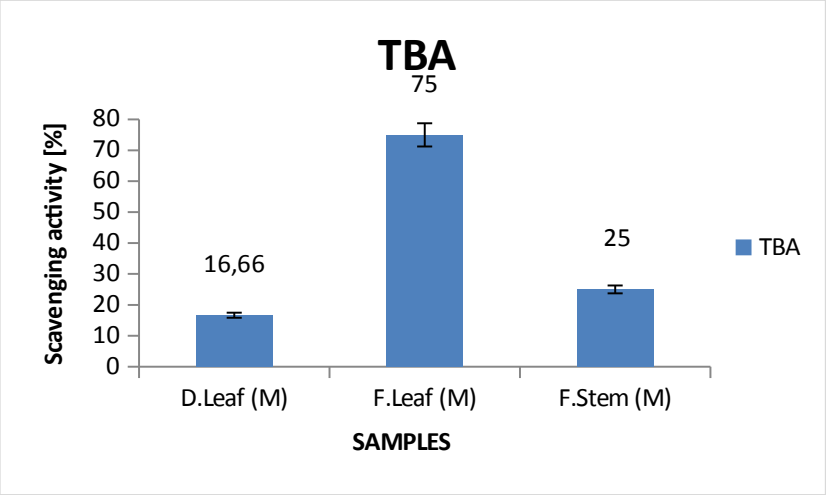

Figure 4 Graphs represents the percentage of scavenging activity [TBA] of *Plectranthus amboinicus* extracts. Each value represents the mean ± SEM; n=3; p<0.05.

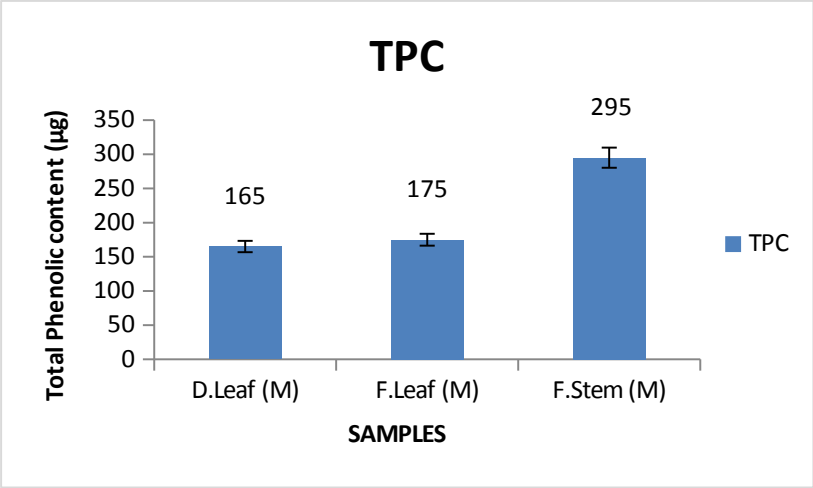

Figure 5 Graphs represents the total phenolic content (µg) of *Plectranthus amboinicus* extracts. Each value represents the mean ± SEM; n=3; p<0.05.

CONCLUSION

The present study concludes the presence of Tannins, Flavonoids, Quinones, Steroids, Phenols, and Reducing sugars in the different plant extracts of *Plectranthus amboinicus*. The extracts were subjected to the total phenol content and also the free radical scavenging activity through the FRAP, FTC and TBA assays. The results showed the presence of high content of phenol and high efficacy of the free radical scavenging activity. The same plant extracts can be used to study further using cell lines and also *in vivo* for their different therapeutic properties. The phenolic content of the stem methanolic extract of *Plectranthus amboinicus* is high than the fresh and dried leaves. Comparative study of the scavenging property through FRAP, FTC and TBA assays of methanolic extract of fresh leaves, dried leaves and fresh stem of *Plectranthus amboinicus* shows the scavenging property is more in methanolic extract of the stem than in fresh and dried leaves. The fresh leaves' methanolic extract has more scavenging ability than the dried leaves. This shows the stem has better activity than fresh and dried leaves.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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