Antioxidant Potency of *Kaempferia galanga* Linn and *Zingiber officinale* var. Rubra rhizomes

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***Abstract*—Reactive oxygen species (ROS) include radical and non-radical oxygen species produced by partial reduction of oxygen. Consumption of antioxidants is necessary to prevent damage to cells, tissues, and organs due to ROS. Red ginger (*Zingiber officinale*) and *kencur* (*Kaempferia galanga*) are known as potential natural antioxidants. The purpose of this research is to determine the potency of antioxidant in ginger red extract (RGE) and *kencur* extract (KE). The extract characteristics were assayed including total phenol content (TPC) using gallic acid standard and total flavonoid content (TFC) using quercetin standard. The antioxidant potency was assayed namely 2,2 diphenyl 1 picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), Nitrogen oxide (NO), 2,2′-Azinobis(3-Ethylbenzthiazoline-6-Sulfonate) (ABTS) scavenging activities, and ferric reducing antioxidant power (FRAP) methods. TPC of RGE and KE were 4.83 and 10.93 µg GAE/mg extract respectively. Meanwhile, the TFC of RGE was 2.68 QE µg/mg extract and KE was 5.67 QE µg/mg extract. The Median Inhibitory Concentration (IC_{50}) in DPPH, ABTS, NO, H_2O_2 scavenging activities of RGE were 79.88; 67.33; 140.35; 212.26 µg/ml respectively and KE were 197.01; 145.16; 52.42; 155.52 µg/ml respectively. The FRAP method shows that KE was greater antioxidant activity compared to RGE. In conclusion, RGE and KE are potential as natural antioxidants.**

***Keywords*—Kempferia galanga Linn, Zingiber officinale, antioxidant, free radical, oxidative stress**

I. Introduction

The radical and non-radical oxygen species that produced by partial reduction of oxygen, such as superoxide anion (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•), are examples of reactive oxygen species (ROS)[1]. The necrosis and apoptosis were due to the damage of nucleic acid bases, lipids, and also proteins. It is the major consequence of oxidative stress, which can seriously impair the function of cell and viability or cause a different of cellular responses via the generation of secondary reactive species [2]. Various diseases are triggered by ROS, mostly inflammation disease and also cancer, atherosclerosis, diabetes, cardiovascular disease, aging, liver diseases. These various diseases are caused by imbalance between free radicals ROS and antioxidants in the body. When free radicals "steal" an electron from a nearby compound or molecule, they produce a new free radical in its place. As a result, the newly created radical seeks to return to its ground state by stealing electrons from cellular structures or molecules with antiparallel spins [3]. The cells are harmed as a result of this chain reaction. The free radical in the body is come from endogenous and exogenous sources. The endogenous sources are come from mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, etc. And the exogenous are mostly come from industrial pollution and also the consumption of alcohol, vegetables that used pesticide and drugs. Besides, smoking with tobacco also cause free radical in the body [4].

Antioxidants are compounds that provide electrons to free radicals, preventing free radical damage to cells. As a result, the molecule is stabilized, avoiding damage to other cells [3]. Consumption of antioxidants can prevent the effect free radical. Previous research has shown that long-term use of synthetic antioxidants can cause skin allergies, gastrointestinal issues, and, in some cases, an increased risk of cancer [5,6,7]. As a result, natural antioxidants are being researched extensively. Natural antioxidants may not have any side effects and are easy to acquire. Natural antioxidants are mainly polyphenol, carotenoids and vitamins [8]. Based on its structure, the hydroxyl groups and phenolic rings are linked to their antioxidant properties.

Red ginger (*Zingiber officinale)* and *kencur* (*Kaempferia galanga* Linn.) belongs to Zingiberaceae family [9]. Both red ginger and *kencur* widely used by the public as spice, drinks and traditional medicine. Red ginger and *kencur* are believed to contain antioxidants, antibacterial, anti-inflammatory

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properties, so they can also be potential as hepatoprotective. This research used standardized extract that produced based on current Herbal Good Manufacturing Practices of National Agency of Drug and Food Control (NA-DFC) Republic of Indonesia. This research was done for preparing novelty Herbal Drug Standardization (Obat Herbal Terstandar – OHT) for hepatoprotective based on antioxidant and anti-inflammatory potency.

II. METHODS

A. Preparation Samples

Red ginger extract (RGE) and *kencur* extract (KE) were obtained from FAST Co. (Depok, West Java, Indonesia) with CoA No. Batch 00103211075, 00103211075. The RGE and KE were extracted from rhizome of red ginger and *kencur* using 70% ethanol solvent with additional substance lactose. The RGE and KE were produced based on current Herbal Good Manufacturing Practices of National Agency of Drug and Food Control (NA-DFC) Republic of Indonesia.

B. Total Phenolic Content and Flavonoid Content

A 15 µl standard gallic acid (Sigma Aldrich, 398225) solution was diluted into 6 concentration level (50.00; 25.00; 12.50; 6.25; 3.13; 1.56 µg/mL). The extracts diluted into concentration of 2000; 1000; and 500 µg/mL for total phenol method. Each standard and also the sample were mixed with 60 µl of Na2CO3 7.5% (Merck, A897992745) and 75 µl Folin- Ciocalteu reagent 10% (Merck, 1.090.010.500) in the microplate. The incubation of solution was conducted at 50 ºC for 10 minutes, then the absorbance was measured at 760 nm of wavelength using a microplate reader (Multiskan Go Reader, Thermo Fisher Scientific 1510). Analysis of the phenol content was carried out based on linear regression equations of the gallic acid equivalent (GAE) (Sigma Aldrich, G7384) [10,11].

The total flavonoid content was measured with an $AlCl_3$ colorimetric assay with minor modification. A 75 µL standard quercetin (Sigma Aldrich, Q4951) solution in 7 concentration level (500.00; 250.00; 125.00; 62.50; 31.25; 15.60; and 7.80 µg/mL) and extracts in the concentration of 2000 and 1000 µg/mL were added to the microplate and mixed with 75 µl of $AlCl_3$ 2% (Merck, 449598). The absorbance of the samples was measured in 415 nm of wavelength with the microplate reader. Analysis of the TFC based on linear regression equations of quercetin equivalent (QE) [11].

C. Determination of 2,2 diphenyl 1 picrylhydrazyl (DPPH) Free Radical Scavenging

Samples of 50 µl (RGE, KE) were mixed with 200 µl of DPPH solution (Sigma Aldrich, D9132). The mixture then incubated in the dark room for 30 min. The absorbance was read using a microplate reader at 517 nm wavelength. The IC_{50} calculation was obtained from the scavenging activity. The equation 1 was the % DPPH of scavenging activity [12,13].

$$\%\ \text{scavenging activity} = \frac{Ac-As}{Ac} \times 100 \quad (1)$$

Ac: The absorbance of negative control solution
As: The absorbance of sample solution

D. FRAP Assay

The modified FRAP method was used. The FRAP reagent was made by 10 mL acetate buffer (pH 3.6), 1 mL FeCl3.6 H_2O in distilled water at 20 mM, and 1 mL 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl. After that, 142.50 µl FRAP reagent and 7.50 µl samples (RGE, KE) were added into 96 well plate then incubated at 37 ^{0}C for 30 min. The absorbance of the mixture was measured at 539 nm by the microplate reader. Following that, a $FeSO_4$ standard curve with varying concentrations was created. The µM Fe (II)/µg extract were used as an unit to presented the result [12,13].

E. ABTS Reducing Activity Assay

The solution of ABTS was made by mixing 14 mM 2,2′-Azinobis(3-Ethylbenzthiazoline-6-Sulfonate) with 4.9 mM $K_2S_2O_8$ (Merck, EM105091), for 16 h at 25°C with the dark condition. The mixture was then diluted in 5.5 mM PBS (pH 7.4) until the absorbance of the mixture solution was 0.70 ± 0.02 at 745 nm. The 2 µl of samples were introduced into microplate of 96 well, followed by 198 µl of ABTS solution. The mixture was then incubated at 30 ^{0}C for 6 min and measured by the microplate reader at 745 nm. ABTS-reducing activity was then used to measure the median inhibitory concentration (IC_{50}). The equation of ABTS reducing activity was calculated with equation 1 [14,15].

F. The Scavenging Activity of Hydrogen Peroxide (H_2O_2)

The 60 µl of samples and blank control were mixed into the microplate then 12 µl of 1 mM Ferrous ammonium sulfate (Sigma Aldrich, 215406). The 63 µl of DMSO (Merck, 1.02952.100) was added to sample's well and 90 µl to control's well followed by 3 µl of H_2O_2 5 mM (Merck, 1.08597.1000). The mixture was incubated at 25°C, in a dark room, for 5 min. 75 µl of 1,10-phenanthroline (Sigma Aldrich, 131377) was then added to the mixture and incubated for 10 min in the dark room at room temperature. The mixture absorbance was then measured by the microplate reader at 510. The scavenging activity of H_2O_2 was calculated by equation 1 [15].

G. The Scavenging Activity of Nitrogen Monoxide (NO)

Samples in various concentrations were mixed with 10 mM sodium nitroprusside (Merck, 106541) in phosphate buffered saline (PBS) (Gibco, 1740576). Then the mixture was incubated at 25°C for 2 h followed by the addition of Griess reagent 1% sulfanilamide (Merck, 111799), 2% H_3PO_4 (Merck, 100573) and 0.1% N-(1-napththyl) ethylenediamine dihydrochloride) (Sigma Aldrich, 222488). The absorbance was carried out with the microplate reader at 546 nm. The scavenging activity of NO was calculated by equation 1 [10].

III. RESULTS

A. Total Phenolic and Flavonoid Content

Determination of total phenolic and flavonoid content of RGE and KE. The linear regression equation for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were equation 5 and 6 respectively.

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$$y = 0.0255x + 0.362 \ (R^2 = 0.9917) \quad (5)$$

$$y = 0.0248x + 0.214 \ (R^2 = 0.9906) \quad (6)$$

The TPC and TFC of RGE and KE as shown in Table I. The TPC and TFC value of KE was greater compared to RGE.

TABLE I. TPC AND TFC OF RGE AND KE

Sample	TPC (GAE µg /mg extract)	TFC (QE µg /mg extract)
RGE	4.83 ± 0.09	2.68 ± 0.46
KE	10.93 ± 0.05	5.67 ± 0.37

Note: The data was given in mean ± SD, n=3

B. DPPH Scavenging Activity

DPPH method was used to determine the scavenging activity of RGE and KE in various concentration. Based on the research that have been done, RGE has greater scavenging activity compared to KE ("Fig.1").

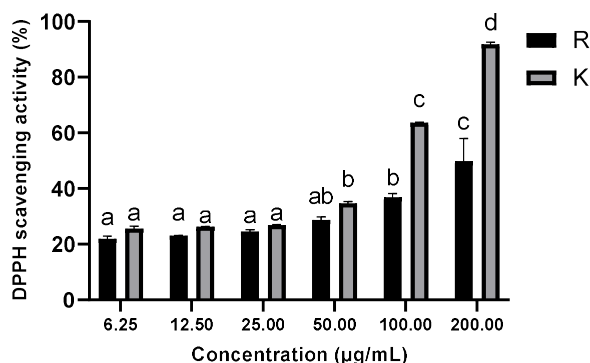

Fig.1. Effect various concentrations of RGE and KE toward DPPH Assay

Note :Data were presented as means ± standard deviation. The differences of alphabetical letter (a, ab, b, c) for RGE and KE show significancy among concentrations at P <0.05 (Tukey HSD post hoc test)

The IC_{50} value of RGE was lower compared to KE (Table II). These results suggest that KE has less antioxidant activity than RGE.

TABLE II. IC_{50} VALUES OF DPPH SCAVENGING ACTIVITIES BY RGE AND KE

Samples	The highest DPPH scavenging activity (%)	Equation	R²	IC_{50} (µg/ml)
RGE	18.99 ± 0.71	y = 0.6725x + 4.7234	0.99	6.77
KE	16.03 ± 2.09	y = 0.2873x + 8.2953	0.99	145.16

Note: The data was given in mean+SD, n=3

C. FRAP

The FRAP activity of RGE and KE as shown in Fig. 2. The result indicates that concentration is equal with FRAP activity.

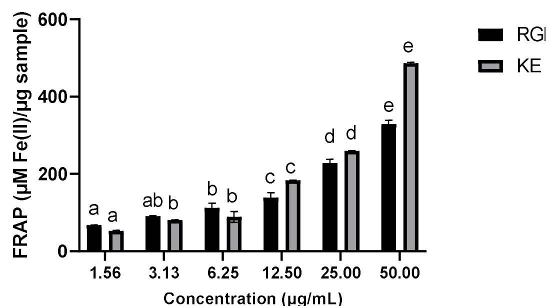

Fig. 2. Effect various concentrations of RGE and KE toward the activity of FRAP

Note :Data were presented as means ± standard deviation. The differences of alphabetical letter (a, ab, b, c) for RGE and KE show significancy among concentrations at P <0.05 (Tukey HSD post hoc test).

D. ABTS Reducing Activity

"Fig. 3" shows the result of ABTS assay. Based on the research that have been done, red ginger has higher ABTS reducing activity compared with *kencur* at the highest concentration.

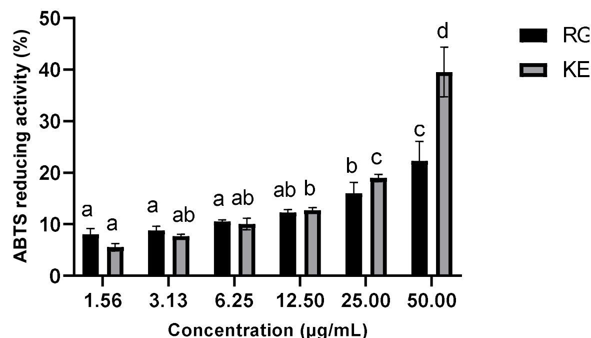

Fig. 3. Effect various concentrations of RGE and KE toward ABTS scavenging activity

Note :Data were presented as means ± standard deviation. The differences of alphabetical letter (a, ab, b, c) for RGE and KE show significancy among concentrations at P <0.05 (Tukey HSD post hoc test)

TABLE III. IC_{50} VALUES OF ABTS REDUCING CAPACITY BY RGE AND KE

Samples	The highest ABTS reducing activity (%)	Equation	R²	IC_{50} (µg/mL)
RGE	39.53 ± 4.82	y = 0.6725x + 4.7234	0.99	67.33
KE	22.86 ± 3.26	y = 0.2873x + 8.2953	0.99	145.16

Note: The data was given in mean ± SD, n=3

"Table III" shows that RGE was more active on ABTS reducing activity compared to KE

E. H_2O_2 Scavenging Activity

Determination of H_2O_2 scavenging activity in various concentration were shows in Fig. 4 and TABLE IV. The result show that *kencur* has higher scavenging activity and lower IC_{50} compared to red ginger. This result indicates that red ginger has less antioxidant activity that *kencur.*

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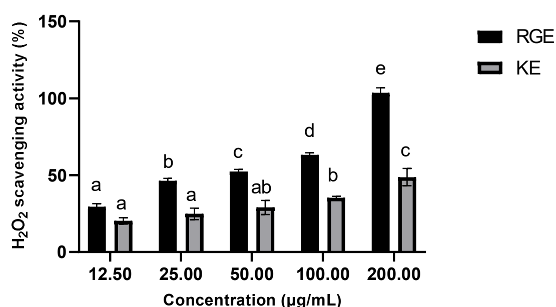

Fig 4. Effect various concentrations of RGE and KE toward H_2O_2 scavenging activity

Note :Data were presented as means ± standard deviation. The differences of alphabetical letter (a, ab, b, c) for RGE and KE show significancy among concentrations at P <0.05 (Tukey HSD post hoc test).

TABLE IV. IC_{50} VALUES OF H_2O_2 SCAVENGING ACTIVITIES BY RGE AND KE

Samples	The highest H_2O_2 scavenging activity (%)	Equation	R^2	IC_{50} (µg/ml)
RGE	48.72 ± 5.72	y = 0.1373x + 20.857	0.99	212.26
KE	103.52 ± 3.31	y = 0.3579x + 31.238	0.97	52.42

Note: The data was given in mean ± SD, n=3

"Table IV" shows that KE was more active on ABTS reducing activity compared to KE.

F. NO Scavenging Activity

NO assay was used to determination of NO scavenging activity in various concentration (Fig. 5). The results show that red ginger has higher NO scavenging activity than *kencur* in highest concentration. The lower IC_{50} indicates the better antioxidants activity. TABLE V shows that red ginger has higher antioxidant activity compared to *kencur.*

TABLE V. IC_{50} VALUES OF NO SCAVENGING ACTIVITIES BY RGE AND KE

Samples	The highest NO scavenging activity (%)	Equation	R^2	IC_{50} (µg/ml)
RGE	46.46 ± 1.60	y = 0.3025x + 7.542	0.99	140.36
KE	45.79 ± 0.04	y = 0.1695x + 23.982	0.98	155.52

Note: The data was given in mean ± SD, n=3

"Table V" shows that KE and RGE were comparable on NO scavenging activity.

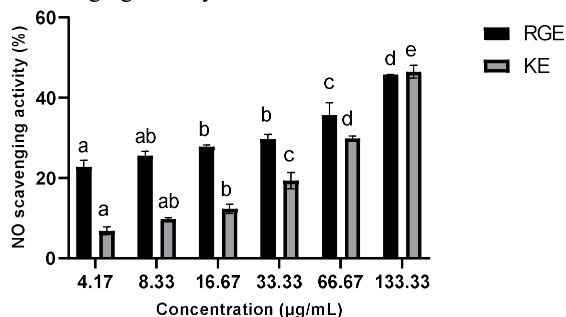

Fig 5. Effect various concentrations of RGE and KE toward NO scavenging activity

This data were presented as means ± standard deviation. The differences of alphabetical letter (a, ab, b, c) for RGE and KE show significancy among concentrations at P <0.05 (Tukey HSD post hoc test).

IV. DISCUSSION

Antioxidant activity of samples were determined by DPPH, FRAP, ABTS, NO and H_2O_2 assay. The DPPH assay was Electron Transfer (ET) antioxidant methods that easy, effective, and rapid way to study plant extract profiles. The technique was no needs sample separation. The FRAP assay also Electron Transfer (ET) antioxidant methods. The technique is speedy, simple, inexpensive, and robust does not required specialized equipment. The FRAP assay can be performed using manual, semiautomated, or automated methods. Nitric oxide (NO) radical inhibition activity; and H_2O_2 and ABTS radical scavenging method are Hydrogen Atom Transfer (HAT) antioxidant methods. Its techniques system in microplate offers a fast and reliable approach.

Phenolic compounds are excellent antioxidants and have a wide range of biochemical functions, including antimutagenic, anticarcinogenic, and gene-expression modification, which may help treat a variety of oxidative stress diseases. The antioxidant activity of phenolic compounds is determined by their composition, the number and location of hydroxyl groups, and the type of substitution on the aromatic ring [16]. Flavonoids are the largest group of naturally occurring phenolic compounds.

TPC was focused on entangling phenolic compounds with a blue complex created by Folin Ciocalteu's reagent. The total polyphenols were calculated using the gallic acid calibration curve [11]. Based on the research that have been done, TPC of RGE and KE were 4.83 and 10.93 µg/mg extract respectively. Meanwhile the TFC of RGE is 2.68 µg/mg extract and KE is 5.67 µg/mg extract. Previous studies have shown that TPC and TFC of RGE were 95.34;53,67 mg/100g extract [17]. Meanwhile the TPC and TFC of methanolic extract of *Kempferia galanga* rhizomes which were 15.40 ± 0.35 mg of gallic acid equivalent/g of extract and 37.72 ± 0.50 mg of catechin equivalent/g of extract, respectively [9] This indicates that RGE has better antioxidant activity compared to KE.

The principle of DPPH, FRAP, NO, ABTS and H_2O_2 methods is color changing that caused by reaction between free radical and antioxidant substance in the samples [18]. The intensity of color change then measured by spectrophotometer. The IC_{50} value is the concentration at which an antioxidant can scavenge 50% of the assay free radical; a lower IC_{50} value indicates greater antioxidant activity.

DPPH is a stable free radical that is reduced when it reacts with a hydrogen donor. In the DPPH assay, antioxidants in the extract are assumed to serve as hydrogen donors, causing DPPH to be reduced. Antioxidants protect against a variety of diseases, including cancer, by scavenging free radicals [19]. The analysis shows that the IC_{50} of RGE is 79.88 µg/mL and KE is 197.01 µg/mL.

The method of FRAP were works by reducing the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-

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triazine) by antioxidants. The Fe^{2+} binding to the ligand results in a deep navy-blue hue [19]. The FRAP method show that *kencur* have Greater antioxidant activity compared to red ginger in highest concentration.

The ABTS assay is commonly used in plant extracts to determine the ability of the extract's compound to scavenge radical activity. As a result of the ABTS cation radical reacting with a hydrogen-donating antioxidant, the solution becomes colorless [19]. Based on the research that have been done, the IC_{50} ABTS reducing activity of RGE is 67.33 μg/mL and KE 145.16 μg/ml.

The analysis of NO assay shown that the IC_{50} of RGE 140.35 μg/mL and KE was 52.42 μg/ml. The based principle of this assay is specific nitric oxide synthases catalyze a biochemical reaction that produces NO in biological tissues [20]. Sodium nitroprusside ($C_5FeN_6Na_2O$), or as known as SNP, reacts with O_2 in buffered saline to produce nitrite ions, and then the measurement was use Griess reagent [21]. The phenanthroline and $(NH_4)_2Fe(SO_4)_2(H_2O)_6$. or as known as ferrous ammonium sulfate reaction were used to assess the activity of antioxidant against hydrogen peroxide (H_2O_2). As phenanthroline combines with ferrous ammonium sulfate, an orange-colored Fe^{2+}-tri-phenanthroline complex forms. The presence of H_2O_2 in the reaction prevents the formation of the complex [22]. The result show that the IC_{50} of RGE is 212.26 μg/ml and KE is 52.42 μg/mL

Red ginger (*Zingiber officinale)* and *kencur* (*Kaempferia galanga* Linn.) belongs to Zingiberaceae family [9] as have been researched, has many biological activities such as antifungal, antioxidant, insecticidal, and anti-inflammatory. Red ginger's antioxidant activity is derived from gingerol, shogaol, and zingerone. Gingerol and shogaol are able to act as primary antioxidants against lipid radicals. Gingerol and shogaol have antioxidant activity because they contain a benzene ring and a hydroxyl group.

Cineol, borneol, 3-carene, camphene, kaempferide, cinnamaldhyde, p-methoxycinnamic acid, ethyl cinnamate, and ethyl-p-methoxy cinnamate have been reported as constituents of Kaempferia galanga rhizomes. [23]. Kaempferol and kaempferide is major flavonoid constituent in *kencur* [24]. Previous studies have shown that kaempferol act as anti-inflammatory, antioxidant, anti-cancer and anti-obesity [25].

The constituent of *Zingiber officinale* and *Kaempferia galanga* are polyphenol compounds. Antioxidant activity values well correlated with polyphenol content [26]. There was also a strong correlation between the methods for measuring antioxidant capacity.

V. CONCLUSION

Based on the research that have been done, the *kencur* extract has greater antioxidant activity in DPPH, ABTS, FRAP and NO method but in H_2O_2 method the red ginger extract has greater antioxidant activity. In conclusion, both red ginger and *kencur* extract are potential as natural antioxidants. It can be used as the active compound, natural additive, and functional food ingredients in various types of pharmaceutical preparations.

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REFERENCES

[1] P. Ray, H. Bo-Wen and T. Yoshiaki, "Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling," Cell Signal, vol. 24, no. 5, pp. 981-990, 2012

[2] M. Gutowski and S. Kowalczyk, "A study of free radical chemistry: their role and pathophysiological significance," Acta Biochimica Polonica, vol. 60, no. 1, pp. 1-16, 2013.

[3] S. Islam, N. Samima, A. K. Mohammad, H. ASM Sakhawat and I. Farhadul, "Evaluation of antioxidant and anticancer properties of the seed extracts of *Syzygium fruticosum* Roxb. growing in Rajshahi, Bangladesh," BMC Complementary and Alternative Medicine, vol. 13, no. 142, pp. 1-10, 2013.

[4] A. Phaniendra, B. J. Dinesh and L. Periyasamy, "Free Radicals: Properties, Sources, TaRGEts, and Their Implication in Various Diseases," Indian Journal of Clinical Biochemistry, vol. 30, no. 1, pp. 11-26, 2015.

[5] W. Wang and K. Kurunthachalam, "Quantitative identification of and exposure to synthetic phenolic antioxidants, including butylated hydroxytoluene, in urine," Enviroment International, vol. 128, pp. 24-29, 2019.

[6] Calleja, B. Lilian, A. Antonio, M. Oliveira and I. Ferreira, "A comparative study between natural and synthetic antioxidants: evaluation of their performance after incorporation into biscuits," Food Chemistry, vol. 216, pp. 342-346, 2017.

[7] S. C. Lourenço, M. Moldão-Martins and V. Alves, "Antioxidants of natural plant origins: from sources to food industry applications," Molecules, vol. 24, no. 22, p. 4132, 2019.

[8] D.-P. Xu, L. Ya, M. Xiao, Z. Tong, Z. Yue, Z. Jie, Z. Jiao-Jiao and L. Hua-Bin, "Natural antioxidants in foods and medicinal plants: extraction, assessment and resources," International Journal of Molecular Sciences, vol. 18, no. 1, pp. 1-22, 2017.

[9] H. Ali, Y. Rumana, S. Mohammed A, H. Rowshanul and Y. Tanzima, "Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. Rhizome against *Ehrlich ascites* carcinoma cells," Journal of King Saud University-Science, vol. 30, no. 3, pp. 386-392, 2018.

[10] S. Utami, Q. R. Sachowardi, N. A. Damayanti, A. Wardhana, I. Syarif, S. Nafik, B. C. Arrahmani, H. S. W. Kusuma and W. Widowati, "Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit as antiaging," Journal of Herbmed Pharmacology, vol. 7, no. 2, pp. 88-93, 2018.

[11] S. Prahastuti, H. Meilinah, T. H. Stella, W. Wahyu, S. W. Wahyu, S. H. Rr.Anisa, R. Rizal and S. W. K. Hanna, "The ethanol extract of the bastard cedar (*Guazuma ulmifolia* L.)," Pharmaciana, vol. 10, no. 1, pp. 77-88, 2020.

[12] W. Widowati, T. L. Wargasetia, E. Afifah and T. Mozef, "Antioxidant and antidiabetic potential of curcuma," Asian Journal of Agriculture and Biology, vol. 6, no. 2, pp. 149-61, 2018.

[13] W. Widowati, B. W. Janeva, S. Nadya, A. Amalia, S. Arumwardana, H. S. W. Kusuma and Y. Arinta, "Antioxidant and antiaging activities of *Jasminum sambac* extract, and its compounds," Journal of Reports in Pharmaceutical Sciences, vol. 7, no. 3, pp. 270-285, 2018.

[14] W. Widowati, A. P. Rani, R. Hamzah, S. Arumwardana, E. Afifah, H. S. W. Kusuma, D. D. Rihibiba, H. Nufus and A. Amalia, "Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds," Natural Product Sciences, vol. 23, no. 3, pp. 192-200, 2017.

[15] C. N. Ginting, I. N. E. Lister, E. Girsang, D. Riastawati, H. S. W. Kusuma and W. Widowati, "Antioxidant activities of *Ficus elastica* leaves ethanol extract and its compounds," Molecular and Cellular Biomedical Sciences, vol. 4, no. 1, pp. 27-33, 2020.

[16] B. Duraiswamy, M. Singanan and V. Vanitha, "Physicochemical, phytochemicals and antioxidant evaluation of *Guazoma ulmifolia*

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Fruit," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 5, no. 5, p. 87, 2018.

[17] G. Oboh, J. Ayodele and O. Adedayo, "Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on Fe^{2+} induced lipid peroxidation in rat brain in vitro. Experimental and Toxicologic Pathology, 64(1-2), 31-36.," Experimental and Toxicologic Pathology, vol. 64, no. 1-2, pp. 31-36, 2012.

[18] I. Gulcin, "Antioxidants and antioxidant methods: an updated overview," Archives of Toxicology, 2020.

[19] A. M. Pisoschi and G. P. Negulescu, "Methods for total antioxidant activity determination: A Review," Biochemistry & Analitycal Biochemistry, vol. 1, no. 1, pp. 1-10, 2011.

[20] M. N. Alam, J. B. Nusrat and R. Muhammad, "Review in vivo and in vitro methods evaluation of antioxidant activity," Saudi Pharmaceutical Journal, vol. 21, no. 2, pp. 143-152, 2013.

[21] P. R. R. Sre, S. Toda and M. Kandasamy, "Phytochemical screening and "in–vitro" anti–oxidant activity of methanolic root extract of *Erythrina indica*," Asian Pacific Journal of Tropical Biomedicine, vol. 2, no. 3, pp. S1696-S1700, 2012.

[22] S. Stevenie, G. Ermy, N. N. Ali and E. L. I Nyoman, "Comparison Activities of peel and extract of lime (*Citrus amblycarpa*) as antioxidant and antielastase," American Scientific Research Journal for Engineering,Technology, and Sciences, vol. 57, no. 1, pp. 77-84, 2019.

[23] H. N, S. Shashidhara, R. P.E and Rajendra, "Comparative evaluation of antimicrobial and antioxidant activities of *Kaempferia Galanga* for natural and micropropagated Plant," International Journal of Pharmacy and Pharmacetical Sciences, vol. 2, no. 4, pp. 73-75, 2010.

[24] A. Kumar, "Phytochemistry, pharmacological activities and uses of traditional medicinal plant *Kaempferia galanga* L.," Journal of Ethnopharmacology, vol. 253, no. 112667, pp. 1-19, 2020.

[25] Y.J. Lee, H.-S. Choi, M.-J. Seo, H.-J. Jeon, K.-J. Kim and B.-Y. Lee, "Kaempferol suppresses lipid accumulation by inhibiting early adipogenesis in 3T3-L1 cells and zebrafish," Food & Function, vol. 6, no. 8, p. 2824–2833, 2015.

[26] D.K. Pratami, A. Mun'im, A. Sundowo, and M. Sahlan, "Phytochemical profile and antioxidant activity of propolis ethanolic extract from Tetragonula bee," Pharmacognosy Journal, vol. 10, no 1, p. 128–135, 2018.

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