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Chemical composition, phytochemical and antioxidant activity from extract of *Etlingera elatior* flower from Indonesia

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Abstract
The flower of *Etlingera elatior* (torch ginger) is widely used in traditional medicine and as a flavour in food was extracted. Extraction has used solvent of methanol, ethyl acetate, and n-hexane. The yield of extraction in methanol, ethyl acetate and n-hexane were 2.36%, 0.54% and 0.21%. Chemical compositions of *Etlingera elatior* flower from Indonesia were identified by analytical Gas Chromatography-Mass Spectroscopy (GC-MS). Total chemical compositions were thirty nine compounds. The result showed that the components mainly were 1-dodecanol (13.82%), dodecanal (12.10%), and 17-pentatriacontene (10.52%). Qualitative analysis of phytochemical constituents in methanol and ethyl acetate extract were tannins, flavonoids, saponin and steroid. Antioxidant activity of *etlingera elatior* flower from Indonesia was carried out by using 1,1, Diphenyl- 2- Picryl Hydrazine (DPPH) free radical scavenging assay. IC_{50} of both flower extract was calculated. Comparative study showed that *etlingera elatior* in the methanol extract shows higher antioxidant potential (IC_{50} = 21.14 µg/ml) as compared to ethyl acetate extract (IC_{50} = 68.24 µg/ml) against DPPH free radicals.

Keywords: *Etlingera elatior*, chemical composition, phytochemical, antioxidant, DPPH

1. Introduction
Zingiberaceae species are represented throughout the tropical and subtropical regions, mainly Asiatic in distribution. *Etlingera elatior* (Jack) R.M. Smith is native to Sumatra, Indonesia and it has been found in many places throughout Southeast Asia. It is commonly known as "kecombrang" among Indonesia and "Kantan" in Malaysia [1]. Etlingera species are very attractive plants because of their varying shades of pink and red colors of bracts and flowers [2].

Torch ginger (*Etlingera elatior*) is a popular plant in south-east Asia wherein their inflorescences are traditionally used for culinary and medicinal purposes. The inflorescence possessed a unique flavor and aroma. It is used traditionally as flavoring and medicine. Leaves of *E. elatior*, mixed with other aromatic herbs in water, are used by post-parfum women for bathing to remove body odour [3, 4]. In recent years, there has been increasing amount of literature in antioxidants and phytochemistry. *Etlingera elatior* inflorescence is known to have high antioxidant properties. Most of the studies on the antioxidant activities of inflorescence of *E. elatior* were limited to rhizomes and leaves [5-7].

Antioxidant are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. Oxidation of biomolecules can cause generation of free radical in body. Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavonoids [8-10]. Natural antioxidant present in foods have attracted interest because of their safety and potential nutritional and therapeutic effect [11, 12].

Antioxidant interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and hence dietary intake of antioxidant compounds are important. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals [13]. Free radical can also effect food quality; reducing its nutritional content and promoting the development of food deterioration [14].

One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH). The molecule of 1,1-diphenyl-2-picryl-hydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over

the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. One parameter that has been introduced recently for the interpretation of the result from the DPPH method, is the "efficient concentration" or IC_{50} value. This is defined as the concentration of substrate that causes 50% loss of the DPPH activity [15-17].

This free radical stable at room temperature. Reduced in the presence of an antioxidant molecule, giving rise to a colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time [18].

The objectives of this study were to evaluate antioxidant activity of *Etlingera elatior* in methanol and ethyl acetat extract by using DPPH radical scavenging method.

2. Material and Methods

2.1. Plant Material

Samples torch ginger flower (*Etlingera elatior*) were purchased fresh from traditional market, Bogor, Indonesia.

2.2. Sampel Extraction

Fresh *Etlingera elatior* was cleaned and washed with fresh water. Sample cutted into small pieces and ready to use for extraction. 250 gram of fresh *Etlingera elatior* flower was macerated in 1000 mL methanol (ratio 1:4 b/v) for five days. The same procedure used for ethyl acetate and n-hexane solvent.

2.3. Analysis by GC-MS

Dry extract in n-hexane of *Etlingera elatior* flower, 2 gram of extract was diluted in 1 mL ethanol 95%. Solution was filtered and the filtrate was inject to GC-MS. Gas Chromatography-Mass Spectrometry analysis were performed using HP agilent 7890, with a capillary column HP 1 (50 m long x 0.25 µm phase thickness 0.22 mm column diameter). Operating condition at the column was follow: start temperature 60 °C, end temperature 220 °C, head rate 2 k/min, end time 20 min. GC-MS used He as the carried gas. Componen were identified by matching their mass spectra with those recorded in the mass spectral library.

2.4. Preliminary Phytochemical Screening

The extracts of following flower was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures [19, 20].

2.4.1. Test for Tannins: 1 ml of sample was taken in a test tube and then 1 ml of HCl 10% and 1 ml ferric chloride 3 % was added and observed for blue-black colouration. Tannin test can use gelatin 10% and sodium-gelatin and observed of precipitation.

2.4.2. Test for Saponin: Crude extract was mixed 5 ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

2.4.3. Test for Flavonoids: Extract was dilute in 1 ml ethanol 96%, 0, 1 g magnesium and 1 mL HCl was added. The yellow or violet colouration disappeares on standing.

2.4.4. Test for Alkaloids: Extract was diluted in ethanol 96%, HCl 2 N was added. Solution was filtered and the filtrate taken into three test tube. Each of test tube identified by reagent of dragendorff, Mayer, and bouchardat. The positive result is white or yellow colour for Mayer reagent, red colour for dragendorff and brown or black for bouchardat.

2.4.5. Test for Steroids: 2 ml of acetic anhydride was added to 0,5 ml crude extract of plant sample with 2 ml H_2SO_4. The colour change from violet to blue or green in sample indicates the presence of steroids.

2.5. Antioxidant Activity

The antioxidant activity was evaluated by free radical scavenging activity (DPPH) method.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is free radical but stable. DPPH solution is initially violet in color which fades when antioxidants donated hydrogen [12]. The change in color is monitored by spectrophotometer and DPPH free radical scavenging activity is calculated [16].

Stock solution of 0.1 mM DPPH in methanol was made. Test sampel of extract were made at 10, 20, 30, and 40 µg/mL in methanol. The absorbance was measured at 514 nm by spectrophotometer pharmaspec UV-1700 (Shimadzu). After 30 minutes and % scavenging was calculated by the equation.

$$\% \text{ Scavenging} = \frac{(A_0 - A_T)}{A_0} \times 100\%$$

Where, Ao = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC_{50}. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%.

3. Result

3.1. Yield of Extraction

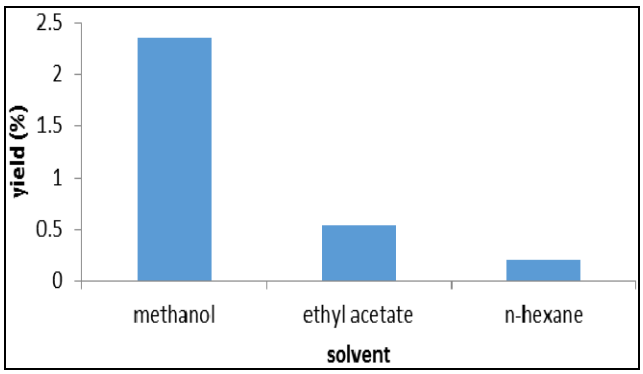

Fig 1: The yield of extraction

3.2. Chemical composition

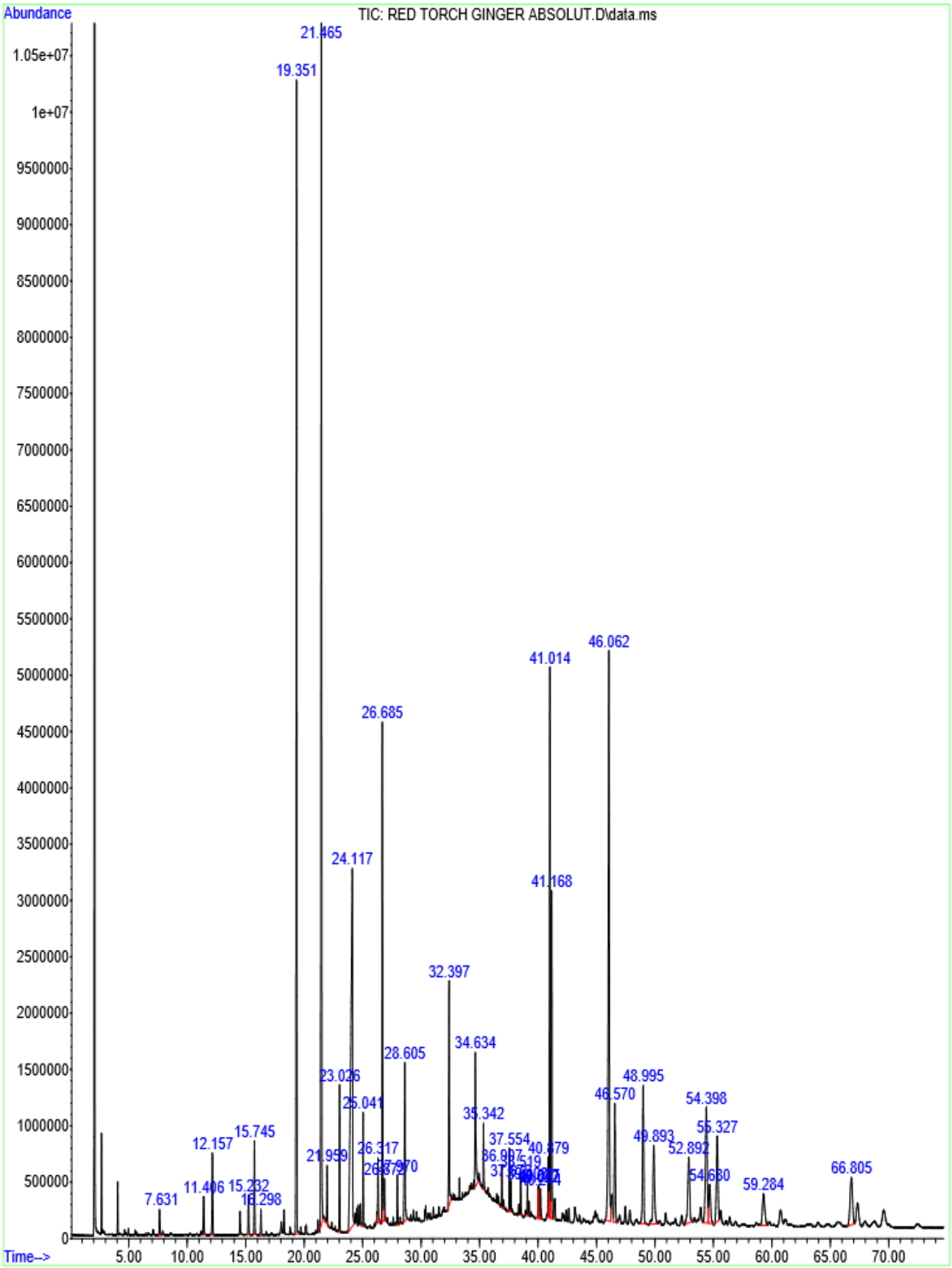

Fig 2: Chromatogram of GC-MS from *Etlingera elatior* flower

Tabel 1: Chemical composition of *Etlingera elatior* flower.

No	Components	Retention time	Percentage
1	Undecane	7.629	0.28
2	Decanal	11.405	0.49
3	Dodecane	12.155	0.98
4	2-undecanone	15.229	0.43
5	Undecanal	15.742	0.99
6	Tridecane	15.741	0.35
7	Dodecanal	19.351	12.10
8	1-Dodecanol	21.463	13.82
9	2-tridecanone	21.960	0.54
10	Cyclododecane	23.024	1.25
11	Dodecanoic acid	24.117	10.04
12	Cis-9-tetradecen-1-ol	26.319	0.71
13	1-Hexadecanol	26.687	4.91
14	Propanedioic acid	26.870	0.37
15	1-tetradecene	27.967	0.41
16	Tetradecanoic acid	28.604	2.17
17	Cis-13-octadecenoic acid	35.341	0.53
18	9, 12 Octadecadienoic acid	34.633	1.90
19	5-Eicosene	36.907	0.32
20	9-tricosane	37.555	0.66
21	Oxirane	38.517	0.36
22	Cyco Tetracosane	39.100	0.33
23	Tridecane	40.024	0.27
24	Icosane	40.078	0.35
25	1,2, benzenedicarboxylic acid	40.231	0.36
26	2-methyl-1-hexadecanol	40.878	0.65
27	1-hexadecene	41.014	6.34
28	1-heneicosyl formate	41.169	3.71
29	17-pentatriacontene	46.062	10.52
30	Cyclotetradecane	46.517	2.10
31	6-nitro-2-metylpyrrolo[2,3] Quinoline	48.977	2.88
32	4-hydrazono-5-hydroxymino-4,5,6,7-tetrahydrobenzofuraxane	49.894	2.09
33	Hexadecanedinitrile	52.892	2.05
34	Cis vaccenic acid	54.339	3.29
35	3-dodecyl cyclohexanone	54.680	1.12
36	Hexadecanoic acid	55.328	2.31
37	Dodecane-1-2-diol O-isopropylidene	59.282	1.21
38	(9E, 12E)-9,12-octa decadienoic acid	66.807	1.86
39	Cholest-5-en-3-ol	78.043	1.31

3.3. Phytochemical Screening

Table 2: Phytochemical screening of *Etlingera elatior* flower

Phytochemicals	Extracts	
	Methanol	Ethyl acetate
Tannins	++	-
Saponins	-	+
Flavonoids	+	++
Steroids	-	+
Alkaloids	-	-

3.4. Antioxidant activity in methanol extract

Table 3: Inhibition control against DPPH at various concentrations in methanol extract

Concentration (µg/ml)	Absorbance	% inhibition
10	0.5889	26.18
20	0.4075	48.92
30	0.2395	69.98
40	0.1128	85.86
A_0 (DPPH solution)	0.7977	

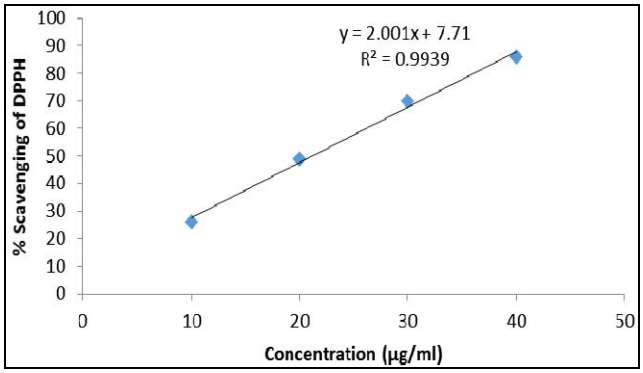

Fig 3: Comparison of % scavenging of DPPH at different concentration in methanol extract.

3.5. Antioxidant activity in ethyl acetate extract

Table 4: Inhibition control of against DPPH at various concentrations in ethyl acetate extract

Concentration (µg/ml)	Absorbance	% inhibition
10	0.6680	19.85
20	0.6348	23.83
30	0.5842	29.90
40	0.5400	35.21
50	0.4958	40.51
A_0 (DPPH solution)	0.8334	

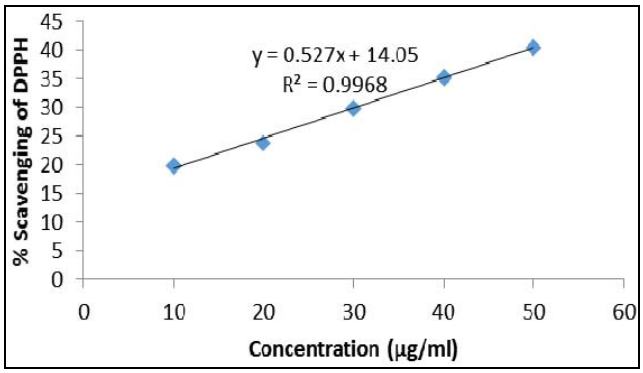

Fig 4: Comparison of % scavenging of DPPH at different concentration in ethyl acetate extract.

Table 5: Inhibition control 50% (IC_{50}) of *Etlingera elatior* extract

Solvent extraction	IC_{50}
methanol	21.14 µg/ml
Ethyl acetate	68.24 µg/ml

4. Discussion

The yield in methanol, ethyl a cetat and n- hexane extract were 2.36%, 0.54% and 0.21% respectively. Differences in yield of extract from different solvent might be attributed to the availability of extractable component of different polarities.

Result GC-MS analysis of torch ginger flower on Fig. 2 shown that the major components were dodecanal a retention time 19.351, 1-dodecanol at retention time 21.463, dedecanoic acid at retention time 24.117, 1-hexadecanol at retention time 26.687, 1-hexadecene at retention time 41.014, and 17-pentatriacontene at retention time 46.062. Analysis by GC-MS of *etlingera elatior* showed that samples contain volatile compounds in n-hexane extract. The torch ginger flower successfully identified thirty nine compounds. The major compounds of torch ginger flower were 1-dodecanol (13.82%), dodecanal (12.10%) and 17-pentatriacontene (10.52%). Other compounds found were dodecanoic acid (10.04%), 1-hexadecene (6.34%), 1-hexadecanol (4.91%), 1-heneicosyl formate (3.71%), cis vaccenic acid (3.29%), hexadecanoic acid (2.31%), cyclotetradecane (2.10%). The other compounds found in minor persentage.

The result of the preliminary phytochemical screening was carried out on the methanol and ethyl acetate extracts of the samples and revealed the presence of a wide range of phytoconstituents including tannins, saponins, flavonoids and steroids as showed in table 2. Phytochemical constituents in methanol extract were flavonoids and tannins and in ethylacetate extract was flavonoids, saponin and steroids. This constituent indicated the antioxidant activities in the sample.

In particular, DPPH radical is widely used for quickly assassing the ability of antioxidants to transfer labile H atoms to radicals [21]. DPPH stable free radical method is an easy, rapid and sensitive to survey the antioxidant activity of a specific compounds are plant extract.

In generally, DPPH scavenging activities increased with increasing phenolic component such as flavonoids, phenolic acids, and phenolic diterpenes. In DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the moleculear a whole. The delocalization also give rise to the deepviolet colour, characterized by an absorption band in methanol solution centered at 517 nm [22].

The DPPH radical scavenging activity in methanol extract and ethyl acetate extract from *Etlingera elatior* were recorded in terms of % inhibition or % scavenging of DPPH as shown in table 3 and table 4. The result showed that the absorbance decrease as a result of a color change from purple to yellow, as the radical was scavenged by anti radicals, through donated of hydrogen to give reduced from DPPH-H. The linear regression found in fig 3 and fig 4. Regression equation is used to determined of IC_{50}.

The ability of *Etlingera elatior* in different extract to donated proton to DPPH free radical is accessed in this assay. Concentration of extract scavenging 50% of DPPH radical shown in table 5. *Etlingera elatior* in methanol extract is potent antioxidant (IC_{50} = 21.14 µg/ml) as compare to ethyl acetate extract (IC_{50} = 68.24 µg/ml).

5. Conclusion

Chemical composition of *Etlingera elatior* showed the components mainly were 1-dodecanol (13.82%), dodecanal (12.10%), and 17-pentatriacontene (10.52%). Research concludes that *Etlingera elatior* from Indonesia in methanol extract shows higher antioxidant potential as compare to ethyl acetate extract.

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7. References

1. Jaavar FM, Osman CP, Ismail NH, Awang K. Analysis of essential oils of leaves, stems, flowers and rhizomes of *Etlingera elatior* (Jack) R.M. Smith. The Malaysia Journal of Analytical Science 2007; 11(1):269-273.
2. Jani NA, Dzakaria N, Pungot NH, Sirat HM. Composition of the essential oils of *Etlingera sphaerocephala* (Baker) C.K. Lim. UMTAS, 2011, 235-239.
3. Wijekoon MMJO, Karim AA, Bhat R. Evaluation of nutritional quality of torch ginger (*Etlingera elatior* Jack) inflorescence. International Food Research Journal 2011; 18(4):1415-1420.
4. Abdelmageed AHA, Faridah QZ, Norhana FMA, Julia AA, Kadir MA. Micropropagation of *Etlingera elatior* (Zingiberaceae) by using axillary bud explants. Journal of Medicinal Plants Research, 5(18), 4465-4469.
5. Jackie T, Haleagrahara N, Chakravarthi S. Antioxidant effects of *Etlingera elatior* flower extract against lead acetate-induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. Biomed Central 2011; 4:67.
6. Yan SW, Asmah R. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. International Food Research Journal 2010; 17:417-423.
7. Chan EWC, Lim YY, Wong SK. Phytochemistry and pharmacological properties of *Etlingera elatior*: A Review. Pharmacognosy Journal 2011; 3(22):69-71.
8. Mandal S, Yadav S, Nema RK. Antioxidant: A Review. Journal of Chemical and Pharmaceutical Research 2009; 1(1):102-104.
9. Lawrence R, Lawrence K. Antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian plains. Asian Pacific Journal of Tropical Biomedicine 2011; 51-54.
10. Janoti DS, Rana M, Rawat AKS. Comparative antioxidant activity of essential oil of leaves of *Citrus limittioides* and *Citrus pseudolimon* of nainital district. Journal of Pharmacognosy and Phytochemistry 2014; 2(5):24-26.
11. Rufino MSM, Fernandes FAN, Alves RE, de Brito ES. Free radical-scavenging behavior of some north-east Brazilian fruits in DPPH system. Food Chemistry 2009; 114:693-695.
12. Subedi A, Amatya MP, Shrestha TM, Mishra SK, Pokhrel BM. Antioxidant and antibacterial activity of methanolic extract of *Machilus odoratissima*. Kathmandu University Journal of Science, Engineering and Technology 2012; 8(1):73-80.
13. Padmanabhan P, Jangle SN. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(2):143-146.
14. Wettasinghe M, Shahidi F. Antioxidant and free radical-scavenging properties of ethanolic extract of defatted

borage (*Borage officinalis* L) seeds. Food Chemistry 1999; 67:399-414.
15. Prakash D, Upadhyay G, Gupta C, Pushpangadan P, Singh KK. Antioxidant and free radical scavenging activities of some promising wild edible fruits. International Food Research Journal 2012; 19(3):1109-1116.
16. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci.Technol 2004; 26(2):211-219.
17. Shahriar M, Hossain MDI, Sharmin FA, Akhter S, Haque MDA, Bhuiyan MA. *In vitro*, antioxidant and free radical scavenging activity of *Withania somnifera* root. Iosr Journal of Pharmacy 2013; 3(2):38-47.
18. Garcia EJ, Oldoni TLC, Alencar SMD, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solution to be applied on bleached teeth. Braz Dent J 2012; 23(1):22-27.
19. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extract. Journal of Pharmacognosy and Phytochemistry 2013; 2(4):22-29.
20. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd., London, 1973, 49-188.
21. Boligon AA, Schwanz TG, de Brom TF, Frohlich JK, Nunes L, Mario DM *et al.* Chemical composition, antioxidant and antimicrobial activity of the essential oil of *Scutia buxifolia* Reissek leaves, Pharmaceutica Analytica Acta 2012; 3(10):1-4.
22. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry 2004; 85:633-640.