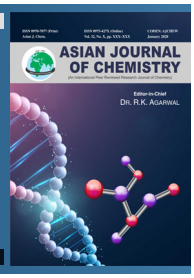
Asian Journal of Chemistry; Vol. 32, No. 5 (2020), 1230-1234

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22257



Preliminary Chemical, Total Polyphenol, Total Flavonoid Contents and Antioxidant Activity of *Eucalyptus camaldulensis* Dehnh.

Nguyen Van Thuy[1,2], Nguyen Minh Tien[3], Nguyen Ngoc Quy[1,2], Mai Huyng Cang[4], Pham Minh Quan[5], Le Minh Bui[2,*], Le Van Minh[6] and Nguyen Van Muoi[7,*]

[1]Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[2]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[3]Deparment of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[4]Department of Chemical Technology, Nong Lam University, Ho Chi Minh City, Viet Nam
[5]Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi City, Vietnam
[6]Research Center of Ginseng and Medicinal Materials, National Institute of Medicinal Materials, Ho Chi Minh City, Vietnam
[7]College of Agriculture, Can Tho University, Can Tho City, Vietnam

*Corresponding authors: E-mail: blminh@ntt.edu.vn; nvmuoi@ctu.edu.vn

Received: 6 June 2019; *Accepted*: 13 February 2020; *Published online*: 29 April 2020; AJC-19861

This study aimed to identify phytochemicals and evaluate antioxidant activities of different extracts from *Eucalyptus camaldulensis*, an aromatic plant in the Myrtaceace family. Examined extracts in different solvents such as diethyl ether extract (DEE), the ethanolic extract (EE) and the aqueous extract (AE). Total polyphenol and total flavonoid contents were determined *via* aluminum chloride colorimetric method. Antioxidant activity was performed *via* ABTS and DPPH radical scavenging assays. The results showed that a wide variety of pharmacologically active compounds such as alkaloid, oil, flavonoid, triterpenoid, saponin, tannin and polyphenol were present in the leaves of *Eucalyptus camaldulensis*. The result of quantitative determination showed that total polyphenol content of the diethyl ether extract, ethanolic extract and aqueous extract achieved 73.47 ± 1.64, 306.48 ± 3.87 and 76.47 ± 1.64 mgGAE/g, respectively. Meanwhile, total flavonoid content was 41.74 ± 2.21, 45.98 ± 1.79 and 18.05 ± 0.81 mgQE/g, respectively. The ethanolic extract exhibited the highest DPPH (IC_{50} = 10.52 ± 0.14 µg/mL) and ABTS (IC_{50} = 9.86 ± 0.17 µg/mL). These results indicate that *Eucalyptus camaldulensis* leaves can be used in dietary applications with the potential to reduce oxidative stress.

Keywords: Antioxidant activity, Total phenolic content, Total flavonoid content, *Eucalyptus camaldulensis* Dehn.

INTRODUCTION

In recent years, medicinal plants have been receiving a great deal of consumer attention due to their health benefit and inability to exert significant side effects to human health. To date, a majority of the world population still depends on plants extracts for primary health care [1-7]. *Eucalyptus camaldulensis* Dehnh. is one of the herb plants that belongs to the family of Myrtaceae which includes approximately 900 species [8]. *Eucalyptus camaldulensis* has been widely cultivated throughout tropical regions and is mainly used as an ingredient for essential oil production [9]. The essential oil extracted from *Eucalyptus camaldulensis* are applied for different purposes such as for treatment of sore throat, treat cold and fever. [10]. Moreover, previous studies showed that *Eucalyptus camaldulensis* possesses a number of pharmacological important activities such as antiviral, antinociceptive, antimicrobial, and antioxidant properties [11-14]. Among them, the antioxidant activity is a characteristic of interest when utilizing a plant into practical applications. This is due to advantages of the natural antioxidant over artificial antioxidants including nontoxicity and ease of decomposition.

Previous study [15] demonstrated that the consumption of natural antioxidants, like phenolics, contributes to the reduced risk of degenerative diseases including cardiovascular diseases and cancer. Similar to essential oils, extracts of *Eucalyptus camaldulensis* leaves are effective natural antioxidants due to the presence of phytochemical compounds existing in the

plants including phenolic and flavonoid composites. Of which, the latter has been highlighted in the literature as the key ingredients responsible for the activity against reactive oxygen species [16-18]. Despite those benefits, flavonoids and polyphenols could not be synthe-sized in the human body, making dietary intakes of the two compounds important for human health. Even though *Eucalyptus camaldulensis* plant has been extensively studied with regards to antioxidant activities and chemical composition, the data on polyphenols and flavonoids of the Vietnamese *Eucalyptus camaldulensis* plant have been lacking. It is given that antioxidant activity of plant largely depends on the variety, extraction method and chemotype [19-21], the present investigation aimed to identify phytochemicals, total polyphenol, total flavonoid and also determine the antioxidant activity of extracts from the *Eucalyptus camaldulensis* plant. The results are expected to contribute to the existing knowledge on the compositions of *Eucalyptus camaldulensis* plant and open up new pathways on utilization of Vietnamese herbals for isolation of useful compounds.

EXPERIMENTAL

Leaves of *Eucalyptus camaldulensis* were obtained at An Giang Province, Vietnam in January 2019. The samples were washed, dried under shade at 40 ºC to remove the water content. The dry samples were ground into fine powders before extraction which was carried out by the procedures described in Fig. 1. Briefly, a powder sample (100 g) was extracted with 1 L of diethyl ether at 25 ºC for 24 h and then concentrated *via* vacuum evaporation to obtain diethyl ether extract (DEE) of *Eucalyptus camaldulensis*. The residue was further extracted with 1 L of 99.5% ethanol and 1 L of distilled water by using the same procedure as above to yield the ethanolic extract (EE) and the aqueous extract (AE), respectively.

Total polyphenol content (TPC): Determination of TPC was performed as previous reported method [17]. First, an extract (0.5 mL) was pipetted with 2.5 mL of Folin-Ciocalteu reagent 10% (v/v). After 5 min, 2 mL of Na_2CO_3 7.5% (w/v) was mixed to the sample. Then, a mixture was vigorously shaken and hatched for 30 min in the dark. In this method, gallic acid acted as a standard. Finally, an absorbance was spectrophotometrically measured at 765 nm and the results were expressed as mg gallic acid equivalents per 1 g of dried extract (mg GAE/g).

Total flavonoid content (TFC): An aluminum chloride colorimetric method was adopted to determine total flavonoid content (TFC) [18]. To be specific, 0.5 mL of extract was added with 0.1 mL 10% $AlCl_3$. Then, 0.1mL of 1M CH_3COOK and 4.3 mL of distilled water were added into the mixture followed by vigorously shaking. In this method, quercetin acted as a standard. An absorbance was spectrophotometrically measured at 415 nm. The results were expressed as mg quercetin equivalents per 1 g of dried extract (mg QE/g).

DPPH scavenging activity: The antioxidant activity of the extracts was evaluated using 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay following a reported method [22]. Briefly, 600 µL of DPPH (O.D. 517 nm = 0.0403 ± 0.013) was introduced into 500 µL of sample solution at different concentrations. The mixture was allowed to stand at room temperature in dark for 30 min. The optical measurement of mixture was performed by UV/VIS-1800 Shimadzu Spectrometer at 517 nm. In this method, 500 µL of EtOH 99.7% acted as blank sample. The standard sample was prepared by dissolving vitamin C (0.1g ÷ 0.01) into EtOH 99.7% in an 100 mL volumetric flask in the

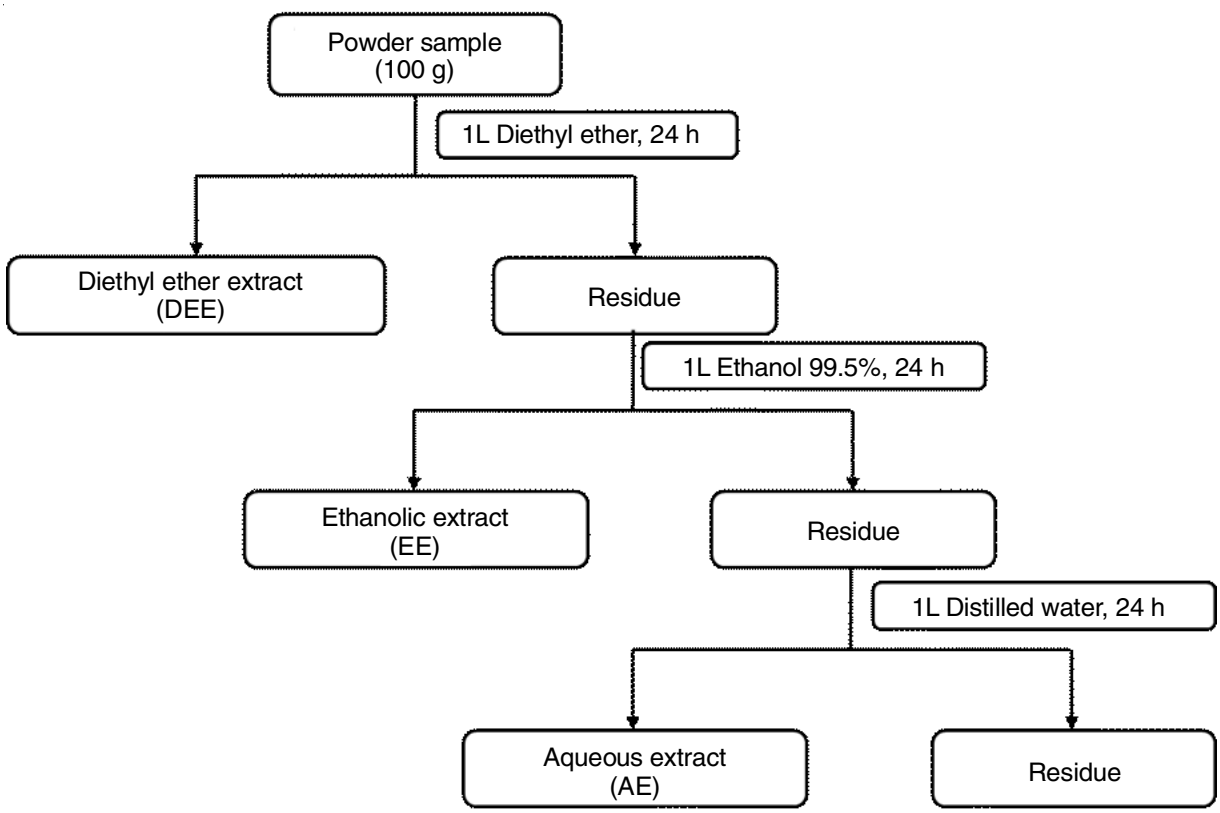


Fig. 1. Extraction scheme of the leaves extracts of *E. camaldulensis* in diethyl ether, ethanol and in aqueous media

dark (C = 100 µL/mL). The percent DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Final results were expressed as IC_{50} indicator, which is the sample concentration required to scavenge 50% of free radicals in DPPH solution.

ABTS scavenging activity: ABTS scavenging activity was determined following a previously described procedure [23]. First, 10 mL of 2.6 mM $K_2S_2O_8$ was added in 10 mL of 7.4 mM ABTS solution, followed by standing for 24 h. Then, a working solution was made by combining 1 mL of stock solution and 60 mL of methanol (O.D. 517 nm = 1.1 ± 0.02). Following that 0.5 mL of sample solution was introduced into 1.5 mL of the working solution, which was allowed to stand for 30 min at room temperature. The mixture was measured for absorbance using UV-VIS spectrophotometer at 734 nm. The percentage of ABTS decolorization of sample was determined as follows:

$$\text{ABTS scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Final results were expressed as IC_{50} indicator, which is the sample concentration required to scavenge 50% of free radicals in ABTS solution.

Statistical analyses: Experiments were performed in triplicate and the results were expressed as mean ± standard deviation. Results of different samples were compared using Tukey's test where statistical significance is recognized as $P < 0.05$. Statgraphics Centurion XV version 15.0 was utilized to produce aforementioned statistical results.

RESULTS AND DISCUSSION

Phytochemical analysis: Used extraction solvent is one of the significant factors which affect extraction efficiency. Table-1 illustrates the phytochemical constituents in different extracts from *Eucalyptus camaldulensis* leaves including diethyl ether extract (DEE), ethanolic extract (EE) and the aqueous extract (AE). Overall, seven components were identified in three *Eucalyptus camaldulensis* extracts which includes oil, alkaloid, flavonoid, trierpenoid, saponin, tannin and polyphenol compounds. In addition, all three extracts shared flavonoids and polyphenols as common compounds and the EE was the extract that could preserve most of the phytochemical classes. Saponin, existed in EE and AE extracts, was also the phytochemical of interest as it showed to confer a strong antimicrobial and tonic properties [24,25].

TABLE-1
PRELIMINARY PHYTOCHEMICAL SCREENING OF THE LEAVES EXTRACTS FROM *E. camaldulensis*

Phytochemical class	Diethyl ether extract	Ethanolic extract	Aqueous extract
Alkaloids	Presence	Presence	Absence
Oils	Presence	Presence	Absence
Tannins	Absence	Presence	Presence
Flavonoids	Presence	Presence	Presence
Triterpenoids	Presence	Presence	Absence
Saponins	Absence	Presence	Presence
Polyphenols	Presence	Presence	Presence
Courmarins	Absence	Absence	Absence

Total polyphenol and total flavonoid contents in different fractions: Fig. 2 illustrates a level of phenolic compounds in diethyl ether (DEE), ethanol (EE) and aqueous (AE) of the leaves extracts from *Eucalyptus camaldulensis*. Among the three extracts,total polyphenol content in EE exhibited the highest content (306.48 ± 3.87 mgGAE/g) followed by AE (76.47 ± 1.64 mgGAE/g) and then in DEE (73.47 ± 1.64 mgGAE/g). These results indicated that leaves of *Eucalyptus camaldulensis* contain a large number of polyphenolic compounds and that the extraction solvent largely determines TPC extracted. For flavonoids, these compounds were one of secondary plant metabolites, playing the key role in anti-inflammatory, anticancer and anti-allergic properties of plant. In *Eucalyptus camaldulensis* extracts, the TFC achieved the highest content in EE (45.98 ± 1.79 mgQE/g), while the TFC in DEE was marginally lower (41.74 ± 2.21 mgQE/g). However, AE had the lowest TFC content (18.05 ± 0.81 mgQE/g), which was approximately 2.5-fold lower than as compared to EE (Table-2).

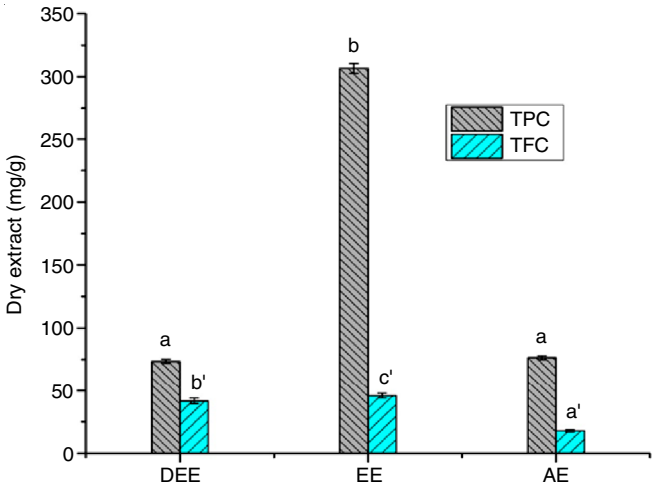


Fig. 2. Total polyphenol content and total flavonoid content in *Eucalyptus camaldulensis* Dehnh

TABLE-2
EXTRACTION YIELDS, TOTAL POLYPHENOL CONTENT AND TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITIES (IC_{50} VALUES) OF *E. camaldulensis* EXTRACTS

Sample	Extraction yields (%)	Total polyphenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	IC_{50} value (µg/mL)	
				DPPH	ABTS
Diethyl ether extract	4.52 ± 0.26^{a}	73.47 ± 1.64^{a}	41.74 ± 2.21^{b}	368.25 ± 1.66^{d}	87.30 ± 1.98^{d}
Ethanolic extract	11.16 ± 0.72^{b}	306.48 ± 3.87^{b}	45.98 ± 1.79^{c}	10.52 ± 0.14^{b}	9.86 ± 0.17^{b}
Aqueous extract	9.05 ± 0.81^{c}	76.47 ± 1.64^{a}	18.05 ± 0.81^{a}	179.75 ± 0.05^{c}	51.17 ± 0.68^{c}
Ascorbic acid	–	–	–	4.80 ± 0.00^{a}	2.66 ± 0.01^{a}

Numbers with identical letters in a column are statistically indifferent ($p < 0.05$)

DPPH radical cation scavenging activity: In this method, antioxidants can remove radicals by hydrogen donation, the antioxidant activity could be determined by observing changes in DPPH absorbance at 517 nm. Fig. 3 illustrates the DPPH radical scavenging ability in DEE, EE and AE fractions of *Eucalyptus camaldulensis* leaves. To be specific, the lowest IC_{50} (10.52 ± 0.14 µg/mL) could be observed in the EE. Meanwhile, the highest IC_{50} belongs to the DEE (368.25 ± 1.66 µg/mL). The results suggested that among *Eucalyptus camaldulensis* extracts, EE exhibited high antioxidant activity.

ABTS radical cation scavenging activity: By observing color reduction at 734 nm, the removal of ABTS radical could be determined. Fig. 4 illustrates the ABTS radical scavenging ability in DEE, EE and AE fractions of *Eucalyptus camaldulensis*

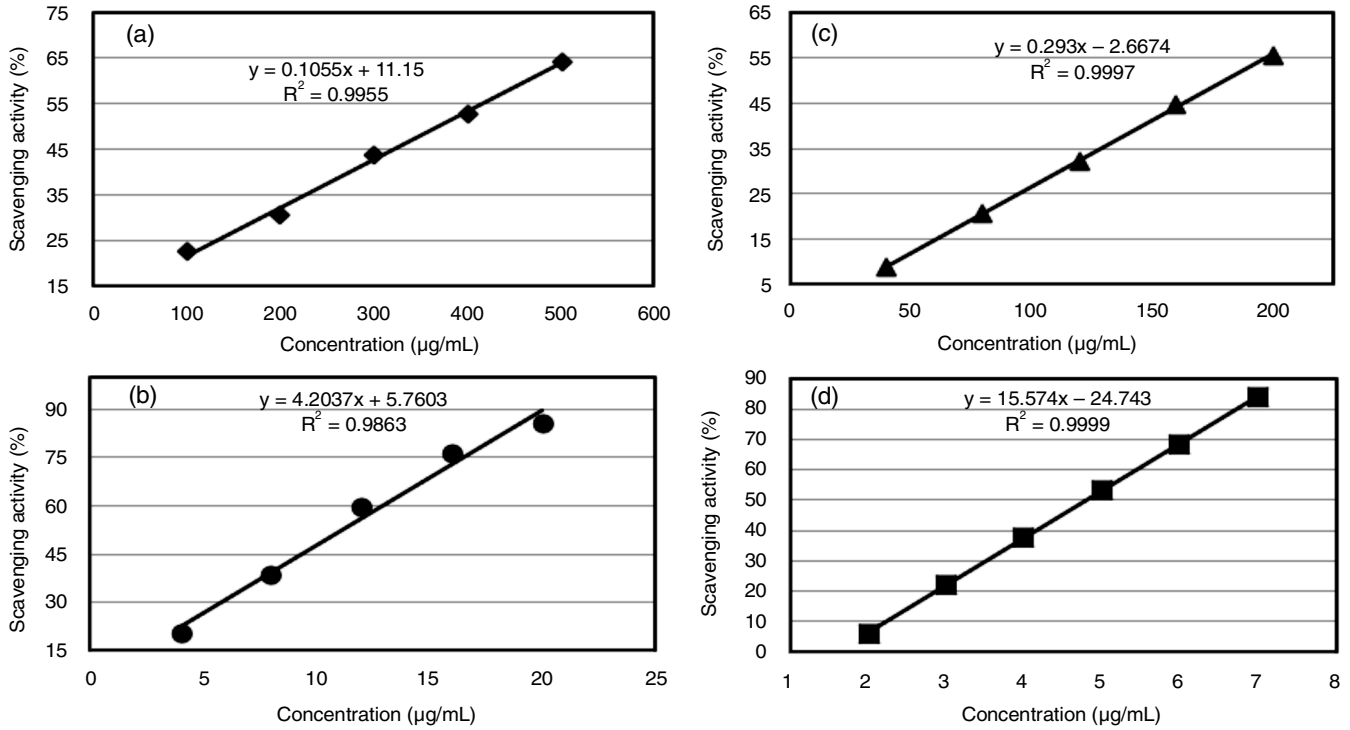


Fig. 3. DPPH radical scavenging activity of dierent extracts from the leaves extracts of *E. camaldulensis*; (a) diethyl ether extract, (b) ethanolic extract, (c) aqueous extract and (d) ascorbic acid

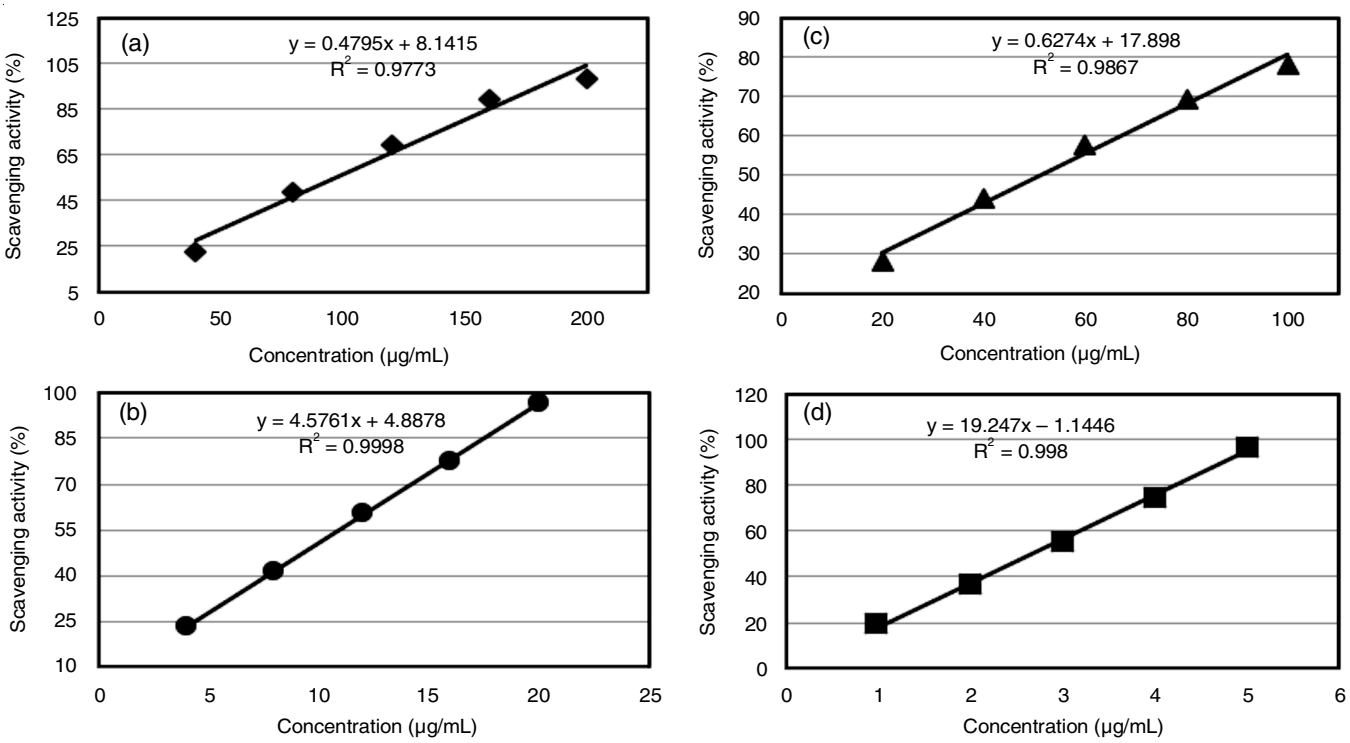


Fig. 4. ABTS radical scavenging activity of dierent extracts from the leaves extracts of *E. camaldulensis*; (a) diethyl ether extract, (b) ethanolic extract, (c) aqueous extract and (d) ascorbic acid

leaves. Similar to the previous assay (DPPH), EE showed the lowest IC_{50} of ABTS radical scavenging (9.86 ± 0.17 µg/mL), while, the highest IC_{50} was of DEE (87.30 ± 1.98 µg/mL).

Conclusion

Present study reports the total content of phenolic, flavonoid and evaluated antioxidant activity of different extracts from *Eucalyptus camaldulensis* leaves. Screening of phytochemicals showed that oil, alkaloid, flavonoid, trierpenoid, saponin, tannin and polyphenols were detected in the extracts. It was found that ethanolic extract of *Eucalyptus camaldulensis* exhibited the highest content of total polyphenols (306.48 ± 3.87 mg GAE/g extract) and flavonoids (45.98 ± 1.79 mgQE/g). For antioxidant activity, ethanolic extract also exhibited lowest IC_{50} values at 306.48 ± 3.87 µg/mL, 9.86 ± 0.17 µg/mL for DPPH and ABTS scavenging activity, respectively. These results suggested that *Eucalyptus camaldulensis* leaves could be a reasonably priced and abundant source of natural antioxidants for food industries.

ACKNOWLEDGEMENTS

This study was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Sofowora, E. Ogunbodede and A. Onayade, *Afr. J. Tradit. Complement Altern. Med.*, **10**, 210 (2013); https://doi.org/10.4314/ajtcam.v10i5.2
2. K. Karunamoorthi, K. Jegajeevanram, J. Vijayalakshmi and E. Mengistie, *J. Evid. Based Complem. Altern. Med.*, **18**, 67 (2013); https://doi.org/10.1177/2156587212460241
3. L. Hoareau and E.J. DaSilva, *Electr. J. Biotechnol.*, **2**, 56 (1999).
4. F. Carmona and A.M.S. Pereira, *Rev. Brasil. Farmacogn.*, **23**, 379 (2013); https://doi.org/10.1590/S0102-695X2013005000018
5. R. Singhal, *J. Health Manag.*, **7**, 277 (2005); https://doi.org/10.1177/097206340500700208
6. M.W. Fowler, *J. Sci. Food Agric.*, **86**, 1797 (2006); https://doi.org/10.1002/jsfa.2598
7. P. Shanley and L. Luz, *BioScience*, **53**, 573 (2003); https://doi.org/10.1641/0006-3568(2003)053[0573:TIOFDO]2.0.CO;2
8. M.I.H. Brooker, J.R. Connors, A.V. Slee and S. Duffy, EUCLID: Eucalyptus of Southern Australia (CD Rom), CSIRO Publishing: Collingwood, Australia (2002).
9. L.J. Bren and N.L. Gibbs, *Aust. Res.*, **16**, 357 (1986).
10. J.P. Essien and E.J. Akpan, *Glob. J. Pure Appl. Sci.*, **10**, 37 (2004); https://doi.org/10.4314/gjpas.v10i1.16355
11. J. Dai and R.J. Mumper, *Molecules*, **15**, 7313 (2010); https://doi.org/10.3390/molecules15107313
12. B.A. Adeniyi, O.O. Ayepola and F.D. Adu, *Pak. J. Pharm. Sci.*, **28**, 1773 (2015).
13. O. Arezki, P. Boxus, C. Kevers and T. Gaspar, *Plant Growth Regul.*, **33**, 215 (2001); https://doi.org/10.1023/A:1017579623170
14. M. Akin, A. Aktumsek and A. Nostro, *Afr. J. Biotechnol.*, **9**, 531 (2010).
15. C. Liapi, G. Anifantis, I. Chinou, A. Kourounakis, S. Theodosopoulos and P. Galanopoulou, *Planta Med.*, **73**, 1247 (2007); https://doi.org/10.1055/s-2007-990224
16. H. Shimoda, J. Tanaka, M. Kikuchi, T. Fukuda, H. Ito, T. Hatano and T. Yoshida, *J. Agric. Food Chem.*, **56**, 4444 (2008); https://doi.org/10.1021/jf8002174
17. L. Bhoopat, S. Srichairatanakool, D. Kanjanapothi, T. Taesotikul, H. Thananchai and T. Bhoopat, *J. Ethnopharmacol.*, **136**, 55 (2011); https://doi.org/10.1016/j.jep.2011.03.061
18. B. Halliwell, J.M.C. Gutteridge and C.E. Cross, *J. Lab. Clin. Med.*, **119**, 598 (1992).
19. D.M. Kasote, S.S. Katyare, M.V. Hegde and H. Bae, *Int. J. Biol. Sci.*, **11**, 982 (2015); https://doi.org/10.7150/ijbs.12096
20. A. Ashraf, R.A. Sarfraz, A. Mahmood and M. Din, *Ind. Crops Prod.*, **74**, 241 (2015); https://doi.org/10.1016/j.indcrop.2015.04.059
21. M.Z.M. Salem, N.A. Ashmawy, H.O. Elansary and A.A. El-Settawy, *Nat. Prod. Res.*, **29**, 681 (2015); https://doi.org/10.1080/14786419.2014.981539
22. H.N.T. Pham, V. Tang Nguyen, Q. Van Vuong, M.C. Bowyer and C.J. Scarlett, *J. Food Process. Preserv.*, **41**, e12879 (2017); https://doi.org/10.1111/jfpp.12879
23. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. Hawkins Byrne, *J. Food Compos. Anal.*, **19**, 669 (2006); https://doi.org/10.1016/j.jfca.2006.01.003
24. G.A. Ayoola, H. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya and E.C. Ezennia, *Trop. J. Pharm. Res.*, **7**, 1019 (2008).
25. G.N. Anyasor, K.O. Ogunwenmo, O.A. Oyelana and B.E. Akpofunure, *Afr. J. Biotechnol.*, **9**, 4880 (2010).