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Evaluation of Antioxidant Activity of *Cinnamomum glanduliferum* Leaf Oil Using Several *In-vitro* Assays

Gina A. Zakaria[a], Nada M. Mostafa[b], Rania F. Abou El-Ezz[a], Omayma A. Eldahshan[b*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Misr International University, Cairo, Egypt*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*

ABSTRACT

Numerous species of the genus *Cinnamomum* have been utilized in traditional medicine for their diverse range of health benefits. One of the key properties of cinnamon that has gained significant attention in recent years is its antioxidant activity. Numerous well-known cinnamon species have been assessed for their antioxidant properties and have exhibited surprisingly positive results, which have prompted further investigation into the potential antioxidant effects of the species under study that have yet to be studied. Cinnamon is classified as a nutraceutical which is a desirable characteristic in the studied plant. In the current study, the essential oil of the leaf of *Cinnamomum glanduliferum* (Wall.) Nees (Lauraceae) was isolated using the Clevenger apparatus and was subjected to GC/MS analysis, enabling the chemical profiling of its constituents. Results revealed that cineole, sabinene, and *α*-terpineol were identified as the prominent compounds in the oil. Our study aimed at examining the antioxidant activity of *Cinnamomum glanduliferum* through the utilization of three widely recognized antioxidant assays: DPPH assay by comparing IC_{50} values of oil with trolox as a standard, FRAP assay by comparing ferrous equivalents of oil with that of ascorbic acid, and ORAC assay by comparing IC_{50} values of oil with quercetin. The results suggest that it may be a promising candidate for further investigation as a potential therapeutic or preventative agent against oxidative stress-related diseases and disorders.

Keywords: *Cinnamomum glanduliferum; Leaf; Volatile oil; Anti-oxidant; DPPH; FRAP; ORAC.*

***Correspondence** | Omayma Eldahshan; Department of *Pharmacognosy*, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt. Email: Oeldahshan@pharma.asu.edu.eg

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1. Introduction

Antioxidants have become an essential aspect of our lives because they can nullify or eliminate ROS (reactive oxygen species) or free radicals. Antioxidants are described as chemical compounds which can attach themselves to free oxygen radicals, thus preventing these radicals from causing harm to healthy cells **[1]**. The body produces free radicals continuously, as oxygen is a vital element for sustaining life. Mitochondria in the cells produce free radicals as by-products when they utilize oxygen to generate energy. These secondary products are generally ROS and RNS (Reactive nitrogen species), arising from cellular redox reactions **[2]**. ROS-induced oxidation leads to the disintegration of cell membranes, damage to membrane proteins, and DNA mutations, which contribute to aging and trigger the progression of various ailments like arteriosclerosis, cancer, diabetes mellitus, liver damage, inflammation, skin disorders, and

coronary heart diseases **[3]**.

According to the World Health Organization, the majority of people worldwide, estimated at 80%, depend on traditional medicine as their main healthcare option, which commonly includes plant extracts and their active components **[2]**.

There are several methods used to measure antioxidant activity based on the mechanisms through which the applied compounds stop chain-breaking reactions. There are two groups: HAT (hydrogen-atom transfer) and SET (single-electron transfer). The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity assay, FRAP (ferric reducing antioxidant power) assay, and CUPRAC (Cupric Reducing Antioxidant Capacity) assays are among the commonly utilized SET methods. Additionally, ORAC (oxygen radical absorbance capacity) assay is included in HAT reaction assays. **[4, 5]**.

Family Lauraceae is a significant group of trees that hold vital importance in the physiognomy of tropical and subtropical ecosystems across the globe. Comprising approximately around 55 genera and 2,500-3,500 species, these trees are primarily found in America and Asia, with a limited number of species occurring in Australia, Madagascar, and Africa **[6]**. Belonging to the family Lauraceae, *Cinnamomum glanduliferum* (Wall.) Nees is widely distributed across India, China, Nepal, Malaysia, Tibet, and Myanmar, and is considered to be native to the Himalayas in India and Nepal **[7]**. It was proven that *Cinnamomum glanduliferum* possessed various biological effects including anti-inflammatory, antimicrobial, anticancer, gastroprotective effects, and many others **[8, 9, 10]**.

Many cinnamon species were reported to have antioxidant activity. It was found that flavonoids from *Cinnamomum camphora* leaf showed a relatively weak anti-oxidant effect at low concentrations, the effect increased with increasing concentration, similar to the positive control of vitamin C **[11]**. Flavonoids from *Cinnamomum septentrionale* showed positive effects on scavenging free radicals, with a concentration of 0.15 mg/mL, it was shown that 86% of DPPH, 68% of hydroxyl radicals, and 59% of superoxide anion free radicals were scavenged **[12]**. In addition, flavonoids found in *Cinnamomum cassia* revealed strong antioxidant activity. The study's findings showed that the purified flavonoids were comparable to vitamin C in their ability to scavenge DPPH free radicals (The IC_{50} values, which were 6.37 g/mL for flavonoids extracted with ethyl acetate, 6.13 g/mL for flavonoids extracted with n-butanol, and 5.07 g/mL for vitamin C, were determined). **[13]**. The success seen with other varieties of cinnamon provides support for the notion of evaluating other species that have yet to be studied.

The current study aims to evaluate the antioxidant activity of *Cinnamomum glanduliferum* leaf oil supported by linking to the activity of certain chemical constituents present in the GC/MS, it is hoped that the positive results will open up new possibilities for the *Cinnamomum glanduliferum* leaf oil to be included as a promising antioxidant drug. In the present era of modern medicine, the need to discover and develop new drugs that can effectively combat a wide variety of ailments has become increasingly important, and the potential of the *Cinnamomum glanduliferum* leaf oil to do that cannot be ignored.

2. Materials and Methods

2.1. Plant Material

In October 2019, *Cinnamomum glanduliferum* (Lauraceae) fresh leaves were obtained from plants cultivated in Al-Zohria

Garden, Cairo, Egypt. A voucher specimen (PHG-P-CG-203) has been stored at the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

2.2. Isolation of volatile oils

The leaves of *Cinnamomum glanduliferum* were cut into small pieces, and the essential oil was extracted from each part using hydrodistillation for 5 h with a Clevenger glass apparatus. The resulting oil was then placed in a glass vial with a screw cap, dried using anhydrous sodium sulfate, and stored in the dark at 4 °C.

2.3. Analysis of oil by GC and GC/MS

GC HP 5890 Hewlett Packard instrument with a flame ionization detector (FID) and an Rtx-5MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) was utilized. A sample volume of 0.03 µL was injected. The oven temperature was programmed to increase from 60 °C to 240 °C at a rate of 3 °C/min. The injector temperature was set at 250 °C, and the detector temperature was maintained at 280 °C. Helium (He) was used as the carrier gas at a flow rate of 1.0 mL/min. An automatic sample injection was performed using 0.02 µL of the oil with a split ratio of 1:70. The relative proportions of the active constituents were determined by calculating the percentages based on peak area normalization.

Additionally, GC/MS analysis was conducted using a PerkinElmer quadrupole MS system (Model 5) coupled with the GC HP 5972, which was equipped with an Rtx-5MS capillary column. The oven temperature was programmed to increase from 45 °C to 240 °C at a rate of 3 °C/min. The injector temperature was set at 250 °C, and the carrier gas, helium (He), flowed at a rate of 0.5 mL/min. An automatic sample injection of 0.02 µL of the oil with a split ratio of 1:70 was employed. The MS operating parameters were as follows: interface temperature: 300 °C, ion source temperature: 200 °C, electron ionization (EI) mode: 70 eV, and the scan range was set from 41 to 400 amu **[14, 15]**.

2.4. Anti-oxidant activity assay (DPPH)

The DPPH Antioxidant Assay Kit from BioVision is a microplate-based assay that enables quick and efficient quantification of the antioxidant capacity in multiple samples. The kit utilizes a deep blue DPPH free radical that abstracts a hydrogen atom, resulting in the formation of pale yellow DPPH, which causes a reduction in absorbance at 517 nm. This change corresponds to the sample's antioxidant capacity.

This assay was performed using a method that has been previously reported **[16]**.

2.5. Anti-oxidant activity assay (FRAP)

The FRAP Assay Kit is a simple and rapid method for assessing the antioxidative capacity of different biological samples. This widely used assay employs antioxidants as reductants in a redox-linked colorimetric reaction, reducing Fe^{3+} to Fe^{2+} ions and forming a colored ferrous-probe complex from a colorless ferric-probe complex at low pH.

This assay was performed using a method that has been previously reported **[17]**.

2.6. Anti-oxidant activity assay (ORAC)

Cell Biolabs' OxiSelect™ ORAC Activity Assay is a rapid and precise technique for evaluating the antioxidant potential of different samples, including cell lysates, plasma, serum, tissue homogenates, and food extracts.

This assay was performed using the method that has been previously reported **[18]**.

3. Results and Discussion

This study revealed noteworthy results, which were effectively presented in a tabulated

form (**Table 1, Fig. 1**). The comparison included two antioxidants - DPPH and trolox, concerning their respective IC_{50} values. The former showed a promising result with an IC_{50} value of 138.2±7.1, while the latter provided an IC_{50} value of 60.78±3.1.

Table 1. Results show different assays confirming the antioxidant activity of oil of *Cinnamomum glanduliferum* leaf

DPPH		FRAP			ORAC	
	IC_{50} μg/mL (mean ± SD)		**mM Ferrous Equivalents ±**	**SD**		**IC_{50} μM TE/L (mean ± SD)**
leaf oil	138.2 ± 7.1	Leaf oil	63.1	2.76	Leaf oil	14.68 ± 1.71
Trolox	60.78 ± 3.1	Ascorbic acid	48.33	2.12	Quercetin	11.8 ± 1.25

DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric ion reducing antioxidant power), ORAC (Oxygen Radical Absorbance Capacity) assays.

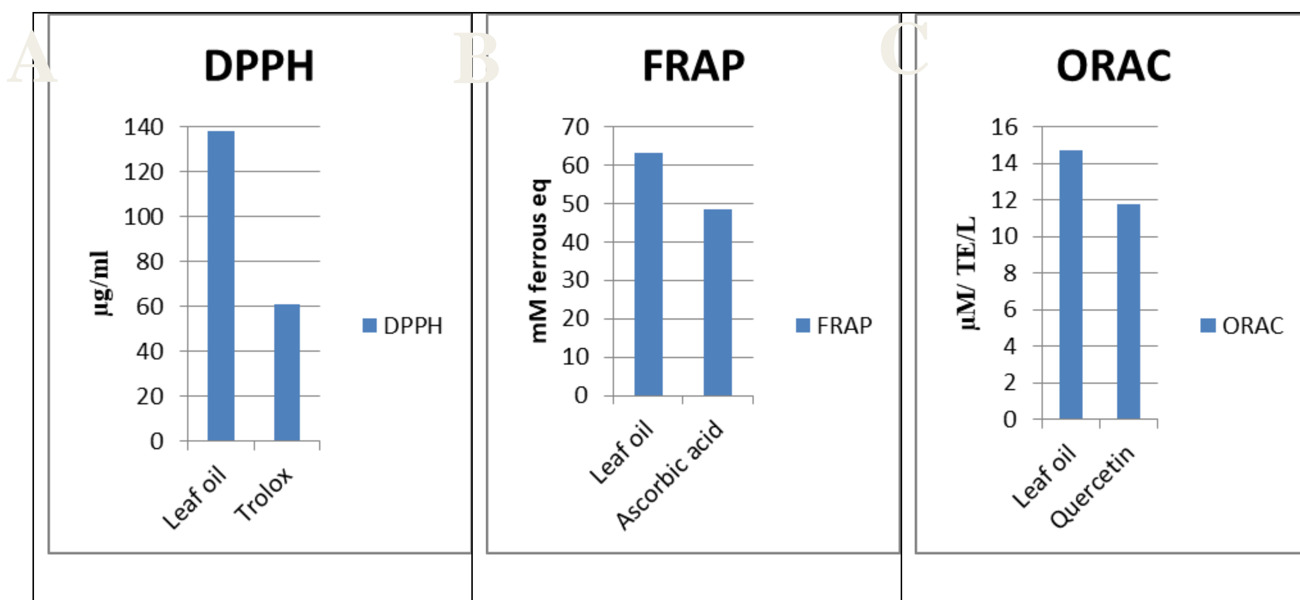

Fig. 1. Diagrams representing each assay **A.** DPPH, **B.** FRAP, and, **C.** ORAC, including leaf oil in comparison with each standard trolox, ascorbic acid, and quercetin respectively. DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric ion reducing antioxidant power), ORAC (Oxygen Radical Absorbance Capacity) assays.

Furthermore, the FRAP assay was carried out, and its results were presented as ferrous equivalents, which were compared with those obtained from ascorbic acid. The leaf oil's FRAP value was seen to be 63.1 mM ferrous equivalent to ascorbic acid, which gave 48.33 mM,

indicating that the leaf oil has a higher ferrous equivalent value.

Additionally, the ORAC assay was conducted, and the results were presented as IC_{50} values, which were compared with those obtained from quercetin. The leaf oil showed promising results with an IC_{50} value of 14.68±1.71, while quercetin gave an IC_{50} value of 11.8±1.25.

Overall, the study's results suggested that the tested antioxidants possessed considerable antioxidant properties. The presented comparison provides valuable insights, which could pave the way for further research on the topic.

In a previously published paper, chemical profiling of *Cinnamomum glanduliferum* leaf oil using gas chromatography was performed **[19]**. The essential oil was found to be rich in various classes of compounds including monoterpenes (oxygenated and hydrocarbons) and sesquiterpenes (oxygenated and hydrocarbons). The studied essential oil was proven to have various biological activities including anti-inflammatory and gastro-protective effects. Only the major compounds identified are listed in **(Table 2)** and the chromatogram is shown in **(Fig. 2)**. Previously, in India, the GC/MS of the essential oil derived from the same genus showed a total of eighteen compounds, with a significant concentration of oxygenated monoterpenes, the compounds 1,8-cineole and *α*-terpineol emerged as the dominant constituents **[20]**.

Also, according to a previous study, the major compounds identified in the leaf essential oil of *Cinnamomum glanduliferum* were cineole, accompanied by linalool, camphor, and *α*-terpineol **[21]**.

In the current GC/MS, The primary chemical constituent that has been identified was found to be eucalyptol which is proven to have a major antioxidant effect, that supports the results obtained from the *in vitro* assays **[22]**. *α*-terpineol was proved to have antioxidant activity and was confirmed using the same assays DPPH and ORAC **[23]**. The current GC/MS results align with previous studies and findings, confirming the presence of all major compounds. This concurrence in results reinforces the notion that the antioxidant effect can be attributed to the activity of these prominent compounds. The consistency between the previous research and the current analysis underscores the significance of these major compounds in exerting antioxidant activity.

Table 2. Listing the major compounds present in the oil of *Cinnamomum glanduliferum* leaf using GC/MS

No	Compounds	Chemical formula	Leaf oil Area (%)	KI
	Eucalyptol (cineole)	$C_{10}H_{18}O$	59.44	1024
2	Sabinene	$C_{10}H_{16}$	14.99	966
3	*α*-Terpineol	$C_{10}H_{18}O$	6.44	1184
4	*α*-Pinene	$C_{10}H_{16}$	5.27	930
5	*β*-Pinene	$C_{10}H_{16}$	3.75	973

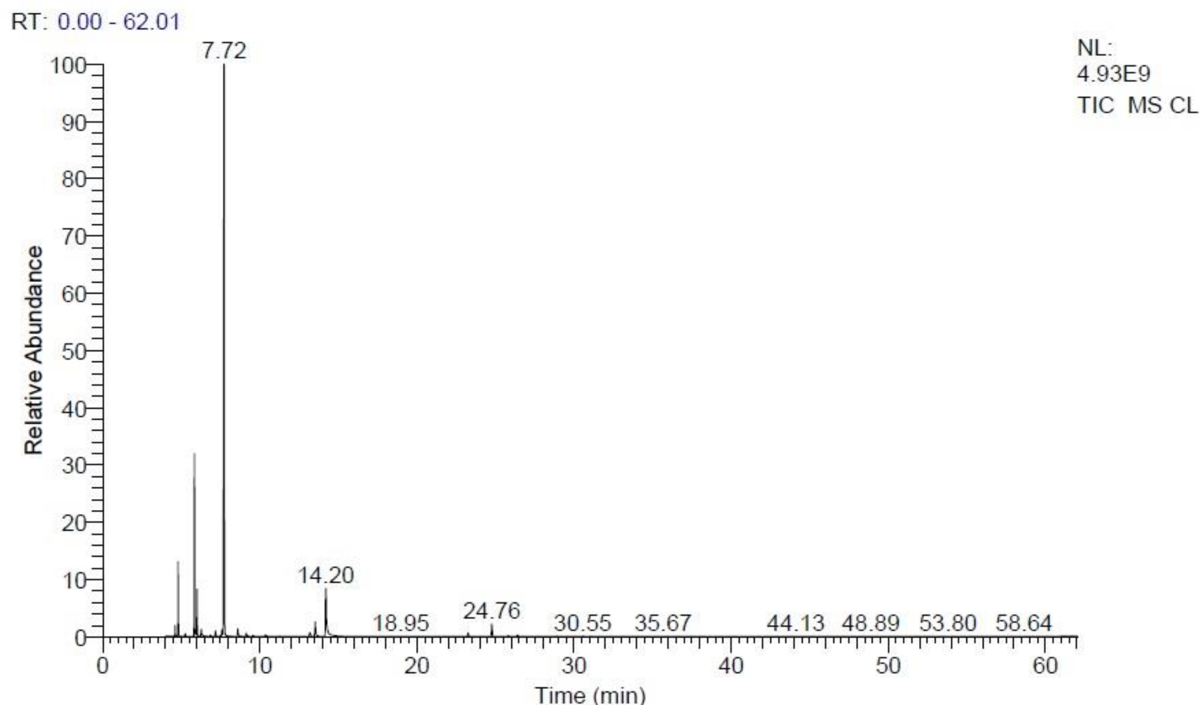

Fig. 2. GC chromatogram of *Cinnamomum glanduliferum* leaf oil.

Conclusion

The *in vitro* studies using DPPH, FRAP, and ORAC assays have demonstrated that the oil derived from C*innamomum glanduliferum* leaf possesses a remarkable antioxidant effect. These findings provide strong evidence for the potential use of *Cinnamomum glanduliferum* leaf essential oil as a natural antioxidant agent in both food and pharmaceutical sectors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and material

All data generated or analyzed during this study were included in the main manuscript.

Competing interest

The authors declare that there are no competing interests.

Funding statement

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Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by [Gina A. Zakaria], [Nada M. Mostafa], [Rania F. Abou El-Ezz], and [Omayma A. Eldahshan]. The first draft of the manuscript was written by [Gina A. Zakaria] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

4. References

1. Dontha, Sunitha. "A review on antioxidant methods." Asian J. Pharm. Clin. Res 9.2 (2016): 14-32.

2. Krishnaiah, Duduku, Rosalam Sarbatly, and Rajesh Nithyanandam. "A review of the antioxidant potential of medicinal plant species." Food and bioproducts processing 89.3 (2011): 217-233. https://doi.org/10.1016/j.fbp.2010.04.008.

3. Karadag, Ayse, Beraat Ozcelik, and Samim Saner. "Review of methods to determine antioxidant capacities." Food anal. methods 2 (2009): 41-60. https://doi.org/10.1007/s12161-008-9067-7.
4. Chaves, Natividad, Antonio Santiago, and Juan Carlos Alías. "Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used." Antioxidants 9.1 (2020): 76. https://doi.org/10.3390/antiox9010076 .
5. El-Nashar, Heba AS, Mariam I. Gamal El-Din, Lucian Hritcu, and Omayma A. Eldahshan. "Insights on the inhibitory power of flavonoids on tyrosinase activity: A survey from 2016 to 2021." Molecules 26, no. 24 (2021): 7546. . https://doi.org/10.3390/molecules26247546.
6. Reis-Avila, Gabriela, and Juliano Morales Oliveira. "Lauraceae: A promising family for the advance of neotropical dendrochronology." Dendrochronologia 44 (2017): 103-116. https://doi.org/10.1016/j.dendro.2017.04.002 .
7. Saxena, Shaiphali. "Traditional utilization and pharmacology of Cinnamomum glanduliferum: a review on current research status." Notulae Scientia Biologicae 14.2 (2022): 11204-11204. https://doi.org/10.55779/nsb14211204
8. Gamal El-Din, Mariam I., Fadia S. Youssef, Mohamed L. Ashour, Omayma A. Eldahshan, and Abdel Nasser B. Singab. "Comparative analysis of volatile constituents of Pachira aquatica Aubl. and Pachira glabra Pasq., their anti-Mycobacterial and anti-Helicobacter pylori activities and their metabolic discrimination using chemometrics." Journal of Essential Oil Bearing Plants 21, no. 6 (2018): 1550-1567.. https://doi.org/10.1080/0972060X.2019.1571950
9. Mostafa, Nada M., Eman A. Abd El-Ghffar, Hoda G. Hegazy, and Omayma A. Eldahshan. "New methoxy flavone from Casimiroa sapota and the biological activities of its leaves extract against lead acetate induced hepatotoxicity in rats." Chemistry & Biodiversity 15, no. 4 (2018): e1700528. https://doi.org/10.1002/cbdv.201700528
10. Kumar, Sanjay, Reshma Kumari, and Shailja Mishra. "Pharmacological properties and their medicinal uses of Cinnamomum: A review." Journal of Pharmacy and Pharmacology 71.12 (2019): 1735-1761. https://doi.org/10.1111/jphp.13173
11. Liu, Zaizhi, Lingtao Kong, Shunbao Lu, and Zhengrong Zou. "Application of a combined homogenate and ultrasonic cavitation system for the efficient extraction of flavonoids from Cinnamomum camphora leaves and evaluation of their antioxidant activity in vitro." Journal of Analytical Methods in Chemistry 2019 (2019). https://doi.org/10.1155/2019/4892635
12. Lu, Hua, Yisheng Zhang, Shengnan Guo, Qiang LI, Yuling LI, and Jinqing LU. "Antioxidant activity of total flavonoids of Cinnamomum Septentrionale Hand.-Mzt." China Pharmacist (2015): 555-557.
13. Wang, Jun, Benzheng Su, Haiqiang Jiang, Ning Cui, Zongyuan Yu, Yuhan Yang, et al. "Traditional uses, phytochemistry and pharmacological activities of the genus Cinnamomum (Lauraceae): A review." Fitoterapia 146 (2020): 104675. https://doi.org/10.1016/j.fitote.2020.104675
14. Ayoub, N., Singab, A. N., Mostafa, N., & Schultze, W. (2010). Volatile constituents of leaves of Ficus carica Linn. grown in Egypt. *Journal of Essential Oil Bearing Plants*, *13*(3), 316-321. https://doi.org/10.1080/0972060X.2010.10643827
15. Ashmawy, Abeer, Nada Mostafa, and Omayma Eldahshan. "GC/MS analysis and molecular profiling of lemon volatile oil against breast cancer." Journal of Essential Oil Bearing Plants 22.4 (2019): 903-916.

https://doi.org/10.1080/0972060X.2019.1667877

16. Uysal, S., Zengin, G., Locatelli, M., Bahadori, M. B., Mocan, A., Bellagamba, G., ... & Aktumsek, A. (2017). Cytotoxic and enzyme inhibitory potential of two Potentilla species (P. speciosa L. and P. reptans Willd.) and their chemical composition. *Frontiers in pharmacology*, *8*, 290. https://doi.org/10.3389/fphar.2017.00290

17. Aly, Shaza H., Omayma A. Eldahshan, Sara T. Al-Rashood, Faizah A. Binjubair, Mahmoud A. El Hassab, Wagdy M. Eldehna, et al. "Chemical Constituents, Antioxidant, and Enzyme Inhibitory Activities Supported by In-Silico Study of n-Hexane Extract and Essential Oil of Guava Leaves." Molecules 27, no. 24 (2022): 8979. https://doi.org/10.3390/molecules27248979

18. Munteanu, Irina Georgiana, and Constantin Apetrei. "Analytical methods used in determining antioxidant activity: A review." International Journal of Molecular Sciences 22.7 (2021): 3380. https://doi.org/10.3390/ijms22073380

19. Azab, Samar S., Gehad A. Abdel Jaleel, and Omayma A. Eldahshan. "Anti-inflammatory and gastroprotective potential of leaf essential oil of Cinnamomum glanduliferum in ethanol-induced rat experimental gastritis." Pharmaceutical biology 55.1 (2017): 1654-1661. https://doi.org/10.1080/13880209.2017.1314512

20. Singh, C., Singh, S., Pande, C., Tewari, G., & Kharkwal, G. C. (2014). Chemical composition of the leaves essential oil from Cinnamomum glanduliferum (Wall) Meissn from Uttarakhand, India. *Journal of Essential Oil Bearing Plants*, *17*(5), 927-930. https://doi.org/10.1080/0972060X.2014.935027

21. Baruah, A., & Nath, S. C. (2006). Leaf essential oils of Cinnamomum glanduliferum (Wall) Meissn and Cinnamomum glaucescens (Nees) Meissn. *Journal of Essential Oil Research*, *18*(2), 200-202. https://doi.org/10.1080/10412905.2006.9699065

22. Xu, Gang, Junsheng Guo, and Chunming Sun. "Eucalyptol ameliorates early brain injury after subarachnoid hemorrhage via antioxidant and anti-inflammatory effects in a rat model." Pharmaceutical Biology 59.1 (2021): 112-118. https://doi.org/10.1080/13880209.2021.1876101

23. Bicas, J. L., I. A. Neri-Numa, A. L. T. G. Ruiz, J. E. De Carvalho, and G. M. Pastore. "Evaluation of the antioxidant and antiproliferative potential of bioflavors." Food and Chemical Toxicology 49, no. 7 (2011): 1610-1615. https://doi.org/10.1016/j.fct.2011.04.012