GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Bioactive Phenolics and Free Radical Scavenging Activity of *Takhtajania perrieri* (Winteraceae)

Ralaivaon-dratsitonta Jumaël Edith Fabrice [2, 4, *], Andrianjato Tartin [1], Andrianarijaona Mamy [2], Rasolofonirina Hubert Xavier [2], Fatiany Pierre Ruphin [2] and Randrianasolo Rivoarison [1]

[1] *Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo.*
[2] *Geosciences, Physics, Environmental Chemistry and High Pathogenic System Doctoral School (GPCEHP), University of Toliara, Toliara 601 Madagascar.*
[3] *Laboratory of Natural Substances Chemistry and Biological Organic Chemistry, Faculty of Sciences, University of Antananarivo, B.P. Box 906, Antananarivo 101, Madagascar.*
[4] *Androy Regional University Center (CURA), University of Toliara, Madagascar.*

GSC Advanced Research and Reviews, 2025, 24(02), 240-245

Publication history: Received on 14 July 2025; revised on 18 August; accepted on 21 August 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.24.2.0253

Abstract

Background: *Takhtajania perrieri* is a critically endangered plant species endemic to Madagascar, belonging to the Winteraceae family. Known as a "living fossil," its rarity and protected status have limited extensive research into its traditional uses. However, its close relatives in the Winteraceae family have been traditionally used to treat ailments like scurvy and stomach issues, suggesting a potential for bioactive compounds within the species.[1]

Aim of the study: This study seeks to determine the phenolic compounds and antioxidant properties of *Takhtajania perrieri* leaves.

Materials and methods: Our team investigated the chemical composition of crude leaf extracts prepared with different solvents: ethanol, hexane, ethyl acetate, and butanol. The isolation of products was achieved through column and thin-layer chromatography. Their structures were elucidated via a combined analysis of 1D and 2D NMR spectra, with confirmation from existing scientific data. Finally, the antioxidant properties of the hexane, ethyl acetate, and butanolic fractions were evaluated using the DPPH° free radical scavenging method.

Results: According to this method, crude ethanolic extract of *Takhtajania perrieri* leaves exhibited an antioxidant capacity (IC_{50}= 2.58 µg / ml), the antioxidant capacity being more powerful than α-tocopherol (11.43 µg / ml), used as standard drug. Its antioxidant capacity is focalised in ethyl acetate extract (IC_{50}= 2.19 µg/ml). The fractionation of the ethyl acetate and butanolic extracts led to the isolation of four phenolic compounds: genistéine (P1), quercetin (P2), 7-hydroxy-4'-methoxyisoflavone(P3) and luteolin (P4).

Keywords: Phenolic compounds; NMR; Antioxidant; *Takhtajania perrieri*

1. Introduction

Takhtajania perrieri is a monotypic genus of flowering plants in the Winteraceae family, represented only by the species *Takhtajania perrieri*. Endemic to Madagascar, it is considered a "living fossil," a term used for ancient plant lineages that have persisted to the present day. This species is restricted to the montane rainforest ecosystems of northern and northeastern Madagascar.[2]

* Corresponding author: Ralaivaon-dratsitonta Jumaël Edith Fabrice

Takhtajania perrieri is considered an extremely rare plant and is classified as critically endangered, which makes it highly significant for conservation programs. Because of its restricted geographic range and legal protection, information on its traditional medicinal or practical applications is scarce. Nevertheless, related species within the same botanical family, like Drimys winteri, have been reported to serve traditional purposes, particularly as stimulants and remedies for different health conditions. [3,4]

To the best of our knowledge, no previous phytochemical and pharmacological investigations have been performed on this species. As a continuation of our study aiming to discover novel chemical structures that can have biological activities from endemic medicinal plants of Madagascar, we carried out a phytochemical investigation of *Takhtajania perrieri* leaves. The fractionation of the ethyl acetate and butanolic extracts led to the isolation of four phenolic compounds. The structural identification of phenolic compounds from this species are described herein.

2. Materials and methods

2.1. General

TLC were performed on aluminium silica gel 60 F254 (Merck) plates (0.2 mm layer thickness). Spots were visualized using UV lamp (254 and 366 nm) and spraying with sulfuric acid reagent. Column chromatography was performed on silica gel 60 (6.3-20 µm) (Merck, Darmstadt, Germany). NMR spectra were recorded with a Brüker AV-400 with a cryoprobe for ^{1}H, ^{13}C RMN. Chemical shift values are in δ (ppm) using the peak signals of the solvent $CDCl_3$ as reference, and coupling constants are reported in Hz.

2.2. Plant material

Takhtajania perrieri leaves were collected from the Andasibe-Mantadia forest in the eastern part of Madagascar in July 2024. Following identification at the herbarium of the Parc Botanique et Zoologique de Tsimbazaza (PBZT) in Antananarivo, a voucher specimen was deposited under the reference [Jum1]. The collected leaves were air-dried at room temperature, then ground into a fine powder, and stored away from light until further use.

2.3. Extraction

The powdered of Takhtajania perrieri leaves (480 g) were extracted with 2000 mL of 90% ethanol at room temperature over 7 days, then filtered. The ethanol was evaporated under reduced pressure to yield 100 g of extract. This ethanolic extract was suspended in warm water (40°C) and subsequently partitioned with hexane, ethyl acetate (AcOEt), and butanol (n-BuOH).

2.4. Phytochemical screening

The detection of main classes of phyto-constituents such as steroids, polysaccharides, flavonoids, saponins, triterpenoids, alkaloids, quinones, leucoanthocyanins, tannins, coumarins, and anthocyanosides were conducted on the crude ethanolic extract according to methods previously published. Appearance of specific colors or precipitates indicates the presence of the targeted metabolites.

2.5. Isolation

The ethyl acetate extract (8 g) was subjected to column chromatography on silica gel (150 g). Elution was carried out isocratically with hexane/AcOEt/MeOH mixtures, first at 50:40:10 and then at 50:35:15 (v/v/v), yielding 200 fractions of 10 mL each. Fractions 8–20 were further purified by preparative TLC using hexane/AcOEt/MeOH (50:40:10, v/v/v) as the mobile phase, affording product P1 (10 mg). Fractions 26–67 were subsequently purified on Sephadex LH-20 with DCM/MeOH (1:1, v/v) as eluent, to isolate compound P2 (15 mg).

In parallel, the butanol extract (8 g) was chromatographed on silica gel (150 g) using hexane/AcOEt/MeOH (10:40:50, v/v/v) under isocratic conditions, giving 13 fractions of 50 mL. Fraction F3 was then purified on Sephadex LH-20 with MeOH as eluent, leading to product P3 (10 mg). Compound 4 (8 mg) was obtained after repeated chromatographic purification of fraction C4.

2.6. Antioxidant assay

- **Qualitative test:** The qualitative antioxidant test was performed using the bioautography method. The extracts were applied to a silica plate, which was then sprayed with a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (2 mg/mL). The presence of an antioxidant product was indicated by a yellow spot on a purple background.

- **Quantitative test:** The quantification of the antioxidant product is carried out according to the method of Brand Williams et al (1995) and Sanchez Moreno et al (1998) with some modifications [5-7] **.** The DPPH (25 mg) is dissolved in 100 ml of methanol. This preparation is stored in the dark. Ten milliliters of this solution are added with 45 ml of methanol. Cascade concentrations of the extract to be tested ranging from (2 mg / ml to 0.125 mg / ml) were prepared. In dry tubes, 200 µl of each concentration were respectively mixed with 3800 µl of the 4.5% DPPH solution. Blanks consisting of 3800 µl of the 4.5% DPPH solution and 200 µl of methanol are also prepared. The test is repeated in triplicate and incubated in the dark for 60 min. The same procedure is applied to the control consisting of vitamin E (α-tocopherol). For the reading, the absorbance is measured using a spectrophotometer at the wavelength of 517 nm. The antioxidant activity which expresses the capacity to scavenge the free radical is estimated by the percentage of discoloration (percentage of inhibition) of DPPH in solution in methanol. The percent inhibition is calculated using formula (1).

$$\text{Percent inhibition (\%)} = \frac{(Ac - As)}{(Ac)} \times 100$$

Ac = the absorbance of the control
As = the absorbance of the sample.

3. Results

3.1. Extraction

Maceration of 480 g of the plant material in ethanol yielded 100 g (20,83%) of crude ethanolic extract. Subsequent maceration using solvents of increasing polarity afforded 10.40 g (2.17%) of hexane extract, 24.50 g (5.10%) of ethyl acetate extract, and 21.43 g (4.46%) of butanol extract.

3.2. Phytochemical screening

The phytochemical screening was performed on the crude ethanolic extract by means of different chemical assays. It revealed the presence of flavonoids, tannins, steroids, terpenoids, leucoanthocyanins, and polysaccharides

3.3. Identification

The structures of the isolates were determined through analysis of their spectroscopic data. The NMR spectra of all isolated compounds are consistent with polyphenol skeletons. By means of 1-D and 2-D NMR spectra and comparison with literature compounds P1,P2,P3 and P4 are recognized as genistéine [8] **,** 7-hydroxy-4'-methoxyisoflavone [9-10], quercetin [11-12]**,** and luteolin respectively (Figure 1).

3.3.1. Genistéine (P1): yellow amorphous powder

^{1}H NMR (600 MHz, $CDCl_3$) δH ppm: ^{1}H NMR (600 MHz, $CDCl_3$) δH ppm: 8.36 (1H, s, H-2); 6.46 (1H, s, H-6); 6.32 (1H, s, H-8); 7.42 (2H, d, J = 8.5 Hz, H-2′, H-6′); 6.86 (2H, d, J = 8.5 Hz, H-3′, H-5′); 12.33 (1H, s, 5-OH); 7.77 (1H, br s, 7-OH) **^{13}C NMR (125 MHz, $CDCl_3$) δC ppm**: 152.6 (C-1); 123.2 (C-2); 182.6 (C-4); 164.9 (C-5); 98.4 (C-6); 165.1 (C-7); 94.8 (C-8); 152.3 (C-9); 105.8 (C-10); 123.0 (C-1′); 130.0 (C-2′, C-6′); 115.2 (C-3′, C-5′); 158.0 (C-4′).

3.3.2. Quercetin (P2): pale yellow powder

δH (ppm) ^{1}H NMR (600 MHz, $CDCl_3$): 7.69 (1H, d, J = 8.4 Hz, H-2′); 7.56 (1H, dd, J = 8.4, 2.0 Hz, H-6′); 6.90 (1H, d, J = 8.4 Hz, H-5′); 6.59 (1H, d, J = 2.0 Hz, H-8); 6.40 (1H, d, J = 2.5 Hz, H-6). **^{13}C NMR (125 MHz, $CDCl_3$) δC ppm:**176.7 (C-4); 164.4 (C-7); 161.6 (C-9); 157.0 (C-5); 148.4 (C-4′); 147.4 (C-2); 145.5 (C-3′); 136.2 (C-3); 122.5 (C-1′); 120.8 (C-6′); 116.1 (C-5′); 115.6 (C-2′); 103.9 (C-10); 99.0 (C-6); 94.0 (C-8)

3.3.3. 7-hydroxy-4'-methoxyisoflavone (P3): yellow amorphous powder

^{1}H NMR (600 MHz, $CDCl_3$) δH ppm: 8.33 (1H, s, H-2); 7.93 (1H, d, J = 8.5 Hz, H-5); 7.57 (2H, d, J = 8.5 Hz, H-2′, H-6′); 6.88 (2H, d, J = 8.5 Hz, H-3′, H-5′); 6.93 (1H, dd, J = 8.0 Hz, H-6); 6.84 (1H, d, J = 2.5 Hz, H-8); 3.87 (3H, s, 4′-OCH_3).

^{13}C NMR (125 MHz, $CDCl_3$) δC ppm: 153.1 (C-2); 163.7 (C-5); 114.3 (C-2′, C-6′); 128.6 (C-3′, C-5′); 98.6 (C-6); 103.1 (C-8); 178.0 (C-4); 163.5 (C-7); 152.4 (C-9); 106.9 (C-10); 124.1 (C-3); 122.6 (C-1′); 157.6 (C-4′); 55.4 (4′-OCH_3).

3.3.4. Luteolin (P4): Garnet powders

δ (ppm) ^{1}H NMR (600 MHz, CD_3OD): δH: 6.72 (1H, d, J = 2.2 Hz, H-2′); 7.14 (1H, dd, J = 8.3, 2.2 Hz, H-6′); 6.89 (1H, d, J = 8.3 Hz, H-5′); 6.70 (1H, s, H-3); 6.30 (1H, d, J = 2.0 Hz, H-8); 6.00 (1H, d, J = 2.0 Hz, H-6).

δ (ppm) ^{13}C NMR (125 MHz, CD_3OD): 182.2 (C-4); 163.8 (C-2); 166.5 (C-7); 161.8 (C-5); 159.0 (C-9); 146.4 (C-4′); 146.0 (C-3′); 122.7 (C-1′); 121.5 (C-6′); 116.3 (C-5′); 115.8 (C-2′); 104.5 (C-10); 105.5 (C-3); 99.0 (C-6); 94.3 (C-8).

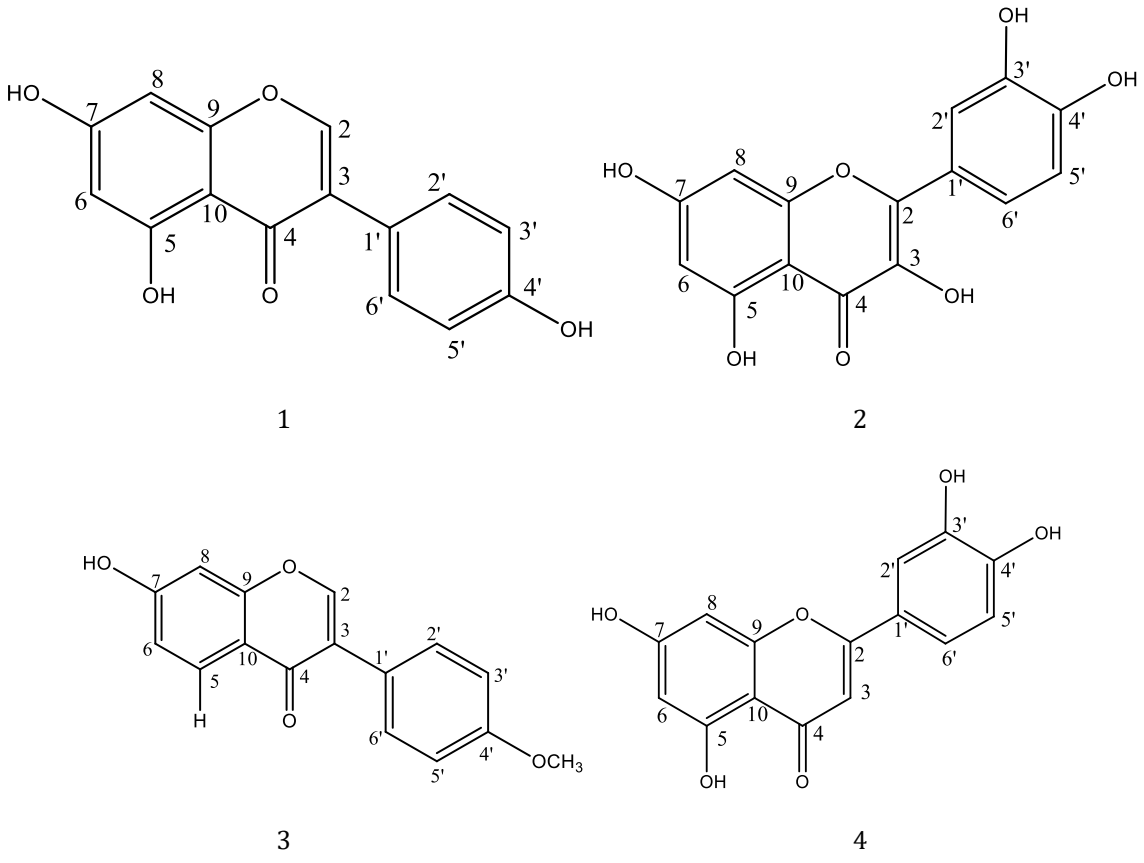
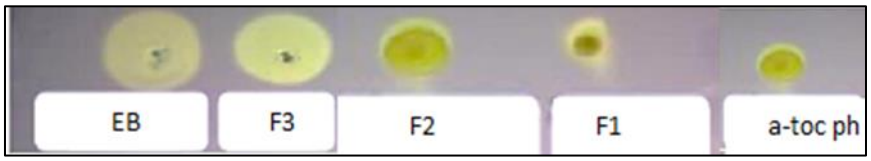


Figure 1 Isolated compounds chemical structures from Takhtajania perrieri (Winteraceae) leaves

3.4. Free radical scavenging activity on DPPH.

The four extracts crude ethanolic (E.B), hexane (F1), ethyl acetate (F2) and butanol (F3) extracts of Takhtajania perrieri leaves, contain antioxidant products (Figure 2).



Stationary phase: Silica gel 60; Developer: DPPH/ MeOH (2 mg/ ml)

Figure 2 Qualitative antioxidant test of Takhtajania perrieri leaves extracts

The DPPH assay is a common method for evaluating antioxidant activity. It uses DPPH° (1,1-diphenyl-2-picrylhydrazyl), a stable free radical with a deep purple color, which absorbs light at 517 nm . When a substance with antioxidant properties is added, it donates a hydrogen atom, neutralizing the DPPH radical and causing the purple color to fade. The change in color intensity is directly related to the substance's antioxidant power. [13-14].

The scavenging abilities of Takhtajania perrieri leaves extracts were concentration dependent, usually expressed as IC_{50} values, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Lower IC_{50} value indicates a higher antioxidant activity. The IC_{50} values of each extract are compared with the IC_{50} value of α-tocopherol (Table 1).

Table 1 Inhibition concentration IC_{50} of the sample tested

Extracts	**IP % à 50 µg/mL**	**IC_{50} (µg/mL)**
Crude extract (E.B)	95,10	2,58 ± 0,15
Ethyl acetate (F2)	95,82	2,19 ± 0,20
n-Buthanol (F3)	75,50	16,23 ± 0,10
α- tocophérol	NT	11,43 ± 0,10

This study evaluated the DPPH radical scavenging activity of various samples, finding the antioxidant capacity to follow this order: ethyl acetate > crude ethanolic > α-tocopherol > butanol. The crude ethanolic extract from the leaves demonstrated a strong antioxidant effect. Based on this analysis, the crude ethanolic extract of Takhtajania perrieri leaves showed a potent antioxidant capacity with an IC50 of 2,58 µg/ml, which is more powerful than the standard drug, α-tocopherol (IC50 = 29,37 µg/ml). The highest antioxidant activity was concentrated in the ethyl acetate extract, with an IC50 of 2,19 µg/ml. The n-butanol fraction was the least active, with an IC50 = 16,23 µg/ml.

4. Discussion

This study's findings indicate that the ethanolic extract of Takhtajania perrieri leaves possesses significant antioxidant properties.

The isolated compounds from the ethyl acetate and butanol extracts, which are common in many plant species, have not been previously reported in the Winteraceae family. While these compounds weren't subjected to bioassays in this study, their biological activities have been documented in prior research**.**

Literature suggests that flavonoids and luteolin are the primary active components responsible for the antioxidant and anti-inflammatory effects observed in many medicinal plants**.** Furthermore, Quercetin is well-known for its potent antioxidant capacity**,** while 7-hydroxy-4'-methoxyisoflavone has been shown to prevent oxidative damage **[17].** Another compound, genistein, has also been reported to have both antioxidant and anti-inflammatory effects [15-19].

5. Conclusion

The results of this study indicate that the leaves of Takhtajania perrieri possess antioxidant activity. This activity is supported by the presence of phenolic compounds that were isolated from the plant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] John, D. H. (2014). The Medicinal Plants of the World: Chemical Constituents, Traditional and Modern Uses. Humana Press.

[2] Schatz, G. E. (2001). Generic Conspectus of the Vascular Plants of Madagascar: A Contribution to the "Flore de Madagascar et des Comores." Missouri Botanical Garden Press. 87(3), 297–302.

[3] Smith, A. B., Jones, C. D., & Miller, E. F. (2001). "Chemical Composition and Antimicrobial Activity of Drimys winteri Bark Extract." Journal of Ethnopharmacology, 75(2-3), 101-108.

[4] Drimys winteri Plants For A Future. (n.d.). Drimys winteri Hook. f. Plants For A Future database. Retrieved August 20, 2025, from https://pfaf.org/user/Plant.aspx?LatinName=Drimys+winteri

[5] Mohamed E.M.I. Abdou., Olivier R., Louise F. Rakotoarison, Stanislas F. Lahadson, Lovasoa Rakotondramasy., Anne W. and Léa H.Rasoanaivo ; Chemical study of Phylloxylon xylophylloides Baker and assessment of its anti-hyperglycaemic activity in Glucose-loaded Normal Rabbits; Journal of Pharmacognosy and Phytochemistry. 2020; 9(2): 2278-2282. http://www.phytojournal.com

[6] Daniela B, Laurian V, Ilioara O, Augustin CM, Grigore D, Daniela H et al. Polyphenolic Composition, Antioxidant and Antibacterial Activities for Two Romanian Subspecies of Achillea distans Waldst. et Kit. ex Willd. Molecules. 2013; 18, 8725-8739. DOI: 10.3390/molecules18088725

[7] Linda Zandi, Marco Makungu, Joan J. E. Munissi, Sandra Duffy, Rakesh Puttreddy, Daniel von der Heiden, Kari Rissanen, Vicky M. Avery, Stephen S. Nyandoro, and Máté Erdélyi, Secoiridoids and Iridoids from Morinda asteroscepa, J. Nat. Prod. 2020, 83, 2641−2646. https://dx.doi.org/10.1021/acs.jnatprod.0c00447

[8] March, R. E., Burns, D. C., & Ellis, D. A. (2007). A predictive tool for assessing ^{13}C NMR chemical shifts of flavonoids. Magnetic Resonance in Chemistry, 45(9), 835–845.

[9] Davis, R. A., & Smith, D. A. (1980). Carbon-13 chemical shift assignments of chromones and isoflavones. Canadian Journal of Chemistry, 58(10), 1095–1102.

[10] Lee, S. H., Lee, J. R., Kim, H. G., Park, D. C., & Kim, H. J. (2016). ^{13}C and ^{1}H NMR assignments of formononetin isolated from Sophora flavescens Ait. Magnetic Resonance in Chemistry, 54(3), 241–244.

[11] Tavakoli, S., Khalighi-Sigaroodi, F., Khosravi Dehaghi, N., Yaghoobi, M., Hajiaghaee, R., Gholami, A., Ghafarzadegan, R. (2022). Isolation and purification of apigenin, quercetin and apigenin 7-O-glycoside from Apium graveolens L., Petroselinum crispum (Mill.) Fuss, Allium cepa L. J. Med. Plants, 21(83), 72-86.

[12] Sinha, D., Biswas, J., & Bishayee, A. (2012). Quercetin, a dietary flavonoid, holds promise for cancer prevention and treatment. In Cancer Therapy (Vol. 10, pp. 121-128). Bentham Science Publishers.

[13] Kandhasamy S, Sun CK. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight and Arn. Saudi Journal of Biological Sciences. 2013; 20, 319–325. https://doi.org/10.1016/j.sjbs.2012.12.005

[14] Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant polyphenols: Chemical properties, biological activities, and synthesis. Angewandte Chemie International Edition, 50(3), 586–621. https://doi.org/10.1002/anie.201000044

[15] Luo, Y., Zhou, W., Chen, J., & Fu, Y. (2020). Luteolin: A review of its antioxidation, anti-inflammatory, and anticancer properties. Journal of Agricultural and Food Chemistry, 68(15), 4165–4181.

[16] Quercetin: Boots, A. W., Haenen, G. R., & Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology, 585(2–3), 325–337. https://doi.org/10.1016/j.ejphar.2008.03.008

[17] Abd-Alla, H. I., Ibrahim, M. T., Taie, H. A., Hasan, M. A., & Shalaby, N. M. (2020). Antioxidant and the efficacy of Sophora secundiflora and methoxyisoflavones in the immune function of pigeons vaccinated against paramyxovirus serotype-1. Pharmacognosy Journal, 12(6), 1276–1288. https://doi.org/10.5530/pj.2020.12.176

[18] Wei, H., & Bowen, R. (2002). Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proceedings of the Society for Experimental Biology and Medicine, 217(3), 386–392. https://doi.org/10.3181/00379727-217-44287

[19] Li, Y., Upadhyay, S., Bhuiyan, M., & Sarkar, F. H. (1999). Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein: Involvement of caspase-3 and Bax. Clinical Cancer Research, 5(11), 3616–3621.