Proceedings of the 1st Botanic Gardens Symposium

Prosiding Simposium Taman Botani Kali Pertama

Strengthening the Networks, Preserving Biological Diversity

Memperkukuh Rangkaian Pemeliharaan Kepelbagian Biologi

- **9 - 10 July, 2024**
- **Faculty of Science and Technology, UKM Bangi 43600 Selangor, Malaysia**

Sponsored by:
Perbadanan Putrajaya

Organized by:
Bangi Botanic Gardens,
FST, UKM

Proceedings of the Ist Botanic Gardens Symposium

Prosiding Simposium Taman Botani Pertama

Strengthening the Networks Preserving Biological Diversity
Memperkukuh Rangkaian Pemeliharaan Kepelbagaian Biologi

***Editors*:**

Noraini Talip
Ahmad Fitri Zohari
Nur Farah Ain Zainee
Hamidun Bunawan
Nur Syazwani Mohd Basir

Taman Botani Bangi, Fakulti Sains dan Teknologi, UKM Bangi

Cetakan Pertama / First Printing, 2025

Diterbitkan di Malaysia oleh / Published in Malaysia by
TAMAN BOTANI BANGI, UNIVERSITI KEBANGSAAN MALAYSIA
43600 UKM Bangi, Selangor Darul Ehsan, MALAYSIA

Penyunting/ Editors
Noraini Talip
Ahmad Fitri Zohari
Nur Farah Ain Zainee
Hamidun Bunawan
Nur Syazwani Mohd Basir

Perpustakaan Negara Malaysia

Penerbit/ Publisher
Taman Botani Bangi
Fakulti Sains dan Teknologi
Universiti Kebangsaan Malaysia
43600 UKM Bangi
Selangor Darul Ehsan
013 – 382 9968

Data-Pengkatalogan-dalam Penerbitan
Cataloguing-in-Publication Data

e ISBN 978-629-94074-0-9

e-Proceedings of the 1st Botanic Gardens Symposium

FOREWORD

We extend our deepest appreciation to all participants, presenters, and researchers who took part in the 1st Botanic Garden Symposium, held under the theme "Strengthening the Networks, Preserving Biological Diversity". This symposium, organized by the Bangi Botanical Garden, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, serves as a vital platform for the sharing of knowledge, experiences, and research findings in the fields of conservation, biodiversity, botany, and botanical garden management at both national and international levels. This symposium brings together academics, researchers, industry practitioners, policy makers, and nature enthusiasts from various backgrounds in a joint effort to strengthen collaborative networks and protect the increasingly threatened biological diversity. It features presentations of the latest research findings, innovative conservation approaches, and best practices in sustainable botanical garden management. The proceedings compile valuable contributions from presenters who have shared discoveries and critical perspectives on the challenges and opportunities that exist today. As an institution committed to sustainable development and the conservation of natural resources, UKM, through the Bangi Botanical Garden, consistently supports initiatives that bridge the gap between science, policy, and society. We hope this symposium serves as a meaningful platform for all parties to strengthen interdisciplinary collaboration, broaden knowledge networks, and nurture greater awareness of the importance of the nation's biodiversity heritage.

PRAKATA

Kami merakamkan setinggi-tinggi penghargaan kepada semua peserta, pembentang, dan penyelidik yang menyertai Simposium Taman Botani Pertama yang bertemakan ″Memperkukuh Jaringan, Memelihara Kepelbagaian Biologi″. Simposium ini dianjurkan oleh Taman Botani Bangi, Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia, sebagai satu wadah perkongsian ilmu, pengalaman dan hasil penyelidikan dalam bidang pemuliharaan, biodiversiti, botani, serta pengurusan taman botani di peringkat nasional dan antarabangsa. Simposium ini menghimpunkan para akademia, penyelidik, pengamal industri, penggubal dasar dan pencinta alam daripada pelbagai latar belakang dalam usaha memperkukuh jaringan kerjasama serta memelihara kepelbagaian biologi yang semakin terancam. Penganjuran ini turut menampilkan pembentangan hasil penyelidikan terkini, pendekatan konservasi inovatif, serta amalan terbaik dalam pengurusan taman botani yang lestari. Prosiding ini menghimpunkan sumbangan berharga para pembentang yang telah berkongsi penemuan dan pandangan kritis terhadap cabaran serta potensi yang wujud pada hari ini. Sebagai sebuah institusi yang bersungguh-sungguh terhadap pembangunan lestari dan pemuliharaan sumber asli, UKM melalui Taman Botani Bangi sentiasa mendukung inisiatif yang mampu merapatkan jurang antara sains, dasar dan masyarakat. Semoga symposium ini dapat menjadi wadah kepada semua pihak dalam memperkukuh kerjasama rentas disiplin, memperluas jaringan pengetahuan serta menyemai kesedaran terhadap kepentingan khazanah kepelbagaian biologi negara. Semoga usaha ini memberi impak berpanjangan dalam memelihara dan memulihara warisan biologi bumi kita.

CONTENT

SCREENING OF PHYTOCHEMICALS, DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS, AND ANTIOXIDANT CHARACTERISTICS OF LEAVES OF *Guioa Diplopetala* (HASSK.) RADLK.

Najwa Omar[1], Norazian Mohd Hassan[2]* & Shamsul Khamis[3]

[1]Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.
[2]Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.
[3]Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
**Corresponding author: norazianmh@iium.edu.my*

ABSTRACT

Guioa diplopetala (Hassk.) Radlk. or Senyamok" is a common shrub of heath forest, and a medicinal herb with antibacterial and antioxidant properties. As there is limited research regarding this plant, hence, this research was carried out to screen the phytochemical compounds, to determine the antioxidant characteristics, total phenolic, and total flavonoid contents of its methanol leaf extract. Simple phytochemical screening was performed to identify the classes of phytochemicals and free radicals DPPH assay method was used to determine the antioxidant activity of the extract. As for the total phenolic and total flavonoid contents, the Folin-Ciocalteu and aluminium chloride methods were used, respectively, whereas thin-layer chromatography (TLC) antioxidant bioautography was carried out to screen for antioxidant active compounds of the extract. Phytochemical screening showed the presence of phenolics, saponins, tannins, flavonoids, terpenoids and steroids in leaves of *G. diplopetala*. The methanol leaf extract also showed an appreciably strong DPPH radical scavenging activity with an IC_{50} value of 4.44 μ/mL and contained a high content of phenolic compounds (4.46 mgCE/gram). TLC bioautography analysis showed the presence of one terpenoid and two phenolics as the main antioxidants with specific TLC characteristics. These results demonstrate that the leaf of *G. diplopetala* is a promising source of natural antioxidants and has the potential to be used in the pharmaceutical industry.

Keywords: Antioxidant activity, bioautography, flavonoids and phenolics, *Guioa diplopetala*, phytochemical screening

INTRODUCTION

Guioa diplopetala (Hassk.) Radlk. or also locally known as "Senyamok" is a shrub or tree that can reach up to 1-1.85 meters in height and is common in the secondary forest of Southeast Asia. It is traditionally used in Indonesia for detoxification and as a treatment for fractured bones (Kristiani et al., 2018). Boiled water extract of the roots is believed to possess anti-inflammatory properties on the mucous membrane (Pratiwi & Nurlaeni, 2022). The leaves possess antioxidant and antibacterial properties and have been positively screened for saponins, alkaloids, flavonoids, anthraquinones, tannins, and terpenoids. However, study and information regarding the medicinal properties of *G. diplopetala* are still lacking. Thus, this study is performed to further analyse the antioxidant active methanol extract of *G. diplopetala* leaves.

Secondary metabolites, also known as phytochemicals, naturally occur in a variety of plant parts, including the fruit, leaf, bark, root, and stem. Among the important phytochemicals are alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes. Even though these compounds do not play a crucial role in plant growth, they are important as a defense against the attacks of herbivores. Besides, they are also significant UV-absorbing substances that shield leaves from harmful light-induced deterioration. Also, they serve as the foundation for numerous pharmaceutical industries as these compounds can be developed into medications that can be used to treat a variety of illnesses (Rabizadeh et al., 2022).

Antioxidants are gaining the attention of people across the globe due to their benefits to the human body. According to Neha et al. (2019), antioxidants are defined as substances that are capable of antagonizing free radicals, thus inhibiting oxidation. They are sometimes called "free radical scavengers" and can be classified as exogenous and endogenous antioxidants. Exogenous antioxidants are commonly found in plants such as vegetables, fruits, spices, and mushrooms. In plants, these natural antioxidants are mostly polyphenols, such as phenolic acids, vitamins, carotenoids, and flavonoids. Furthermore, antioxidants could

be used in the treatment of certain pathophysiological disorders that involve free radicals (Alamzeb et al., 2023).

MATERIALS AND METHODS

Materials

Sodium nitrite, 10% ammonia, 5% ferric chloride solution, mercury (III) chloride, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, Dichloromethane (EMSURE, Germany), sodium hydroxide (BDH, America), DMSO (Uvasol, Germany), DPPH, catechin, concentrated H_2SO_4, methanol (R&M Chemical, Malaysia), aluminium chloride, ascorbic acid (Alfa Aesar, China), TLC plates (Merck KgaA, Germany), flat-bottom 96-well plate (Nest, China),

Methods

Collection of plant materials

The leaves of *G. diplopetala* were acquired from the IIUM Forest of 'Ilm, IIUM Kuantan Campus. The species was identified by Dr. Shamsul Khamis, a taxonomist from UKM B Herbarium, National University of Malaysia. Voucher specimen with the number PIIUM 0355 was deposited at the Herbarium Kulliyyah of Pharmacy, IIUM Kuantan. The leaves were dried using an oven at 50 °C for 7 days and the dried leaves were ground into powder form.

Sample extraction

100 g of dried leaf powder was weighed and macerated in 500 mL of methanol at room temperature overnight. The extract was filtered, and the filtrate was evaporated using a rotary evaporator until it was almost dry. The concentrated extract was transferred into a glass vial and was left in the fume hood for complete removal of solvent before being stored in the freezer at a temperature of 4 °C. The percentage yield (% w/w) for each plant part was calculated as below:

$$\text{Percentage yield} = \frac{\text{weight of dried extract}}{\text{weight of dried powder}} \text{x } 100 \quad \text{- Equation 1}$$

Phytochemical screening of the methanol extract of G. diplopetala leaves

The methanol extract of *G. diplopetala* leaves was screened for the presence of various classes of phytochemicals following the procedures by Iqbal et al. (2015) with slight modifications. The tests include the frothing test for saponins, Mayer's test for alkaloids, lead acetate test for flavonoids, Borntrager's test for anthraquinone derivatives and anthraquinones, ferric chloride test for tannins and phenolic compounds, Salkowski test for terpenoids, Liebermann-Burchard test for sterols and/or triterpenoids and organoleptic test for volatile oils.

DPPH assay for antioxidant activity

Antioxidant activity of the extract was determined by using the DPPH assay following the procedures by Bobo-García et al. (2014) with slight modifications. Eight 2-fold diluted extracts in DMSO were transferred into 96 96-well microplate in triplicate at a concentration range from 100 µg/mL to 0.781 µg/mL. Next, 180 µL of DPPH solution (150 $\mu molL^{-1}$ in methanol: water (80:20, v/v)) was added. The mixture was shaken for 60 seconds and incubated for 40 minutes at room temperature, in the dark. The absorbance was measured at 515 nm by using a microplate reader. Ascorbic acid was used as a standard at eight 2-fold dilutions of 100 $\mu molL^{-1}$. 20 µL of diluted extract/sample at different tested concentrations with 180 µL methanol: water (80:20, v/v) was used as a blank, while 20 µL DMSO with 180 µL DPPH solution was used as a control.

Total phenolic content (TPC)

Total phenolic content was determined according to the procedures by Bobo-García et al. (2014). A total of 20 µL of a two-fold diluted extract was mixed with 100 µL of 1:4 diluted Folin–Ciocalteu reagent in a flat-bottom 96-well microplate. The mixture was shaken for 60 seconds and was left for 240 seconds. Next, 80 µL of sodium carbonate solution was added and the mixture was shaken at

medium-continuous speed for 1 min. After that, the mixture was incubated for 2 hours at room temperature. Then, the absorbance was measured at 750 nm by using the microplate reader. The blank for this assay includes blank extract, which was 20 µl of extract (at each two-fold diluted concentration tested) and 180 µl water, and DMSO instead of extract in the same reaction mixture. Lastly, gallic acid dilutions (final concentration range of 3.205 – 102 mgL^{-1}) were used as standards for calibration.

Total flavonoid content (TFC)

The total flavonoid content was determined according to Herald et al. (2012). 100 µL distilled water was added to each of the 96 wells, then was followed by 10 µL of 50 gL^{-1} $NaNO_2$ and 25 µL of the sample solution. The mixture was incubated for 5 minutes, followed by adding the 15 µL of 100 gL^{-1} $AlCl_3$ to the mixture and then incubated for another 6 minutes before being added with 50 µL of 1 $molL^{-1}$ NaOH and 50 µL of distilled water. The absorbance was measured at 510 nm. The blanks that were used for this assay were mixtures of 25 µL extract at different tested concentrations and 225 µL water, and another blank contained 25 µL DMSO, which replaced the extract and the other reaction mixture as previously mentioned. Catechin was used at a final concentration range of 9.375 μgmL^{-1} – 300 μgmL^{-1} to generate a calibration curve.

Thin-layer chromatography (TLC) antioxidant bioautographic assay

TLC analysis was performed on silica gel plates with a size of 5 x 10 cm. The extract was dissolved in methanol at a concentration of 5 mg/mL and spotted in duplicate at the origin of each plate by using a capillary tube. The TLC plates were developed in a TLC chamber containing 20 mL of 100% dichloromethane and dichloromethane: methanol (9:1) as the solvent systems. The plates were separately visualised by viewing under visible light, UV lights (254 and 366 nm), spraying with ferric chloride, and vanillin/sulphuric acid staining reagents. Bioautography screening for the antioxidant was conducted by spraying the chromatogram with 0.05% DPPH reagent in methanol. Next, the R_f x 100 value of detected major compound(s) from the extract was calculated by using the following equation:

$$R_f \times 100 = \frac{X(\text{distance of the detected spot from the origin})}{Y(\text{distance of solvent front from origin})} \times 100 \quad \text{- Equation 2}$$

RESULTS AND DISCUSSION

Phytochemical screening

Table 1 shows that the methanol extract of *G. diplopetala* leaves contains phenolics, tannins and flavonoids in relatively higher abundance than terpenoids, saponins and steroids. Previously, alkaloids and anthraquinones were detected (Kristiani et al., 2018) while saponins were present in moderate amounts (Pratiwi & Nurlaeni, 2022) in the leaf samples from Indonesia. This difference might be a result of the influence of geographical factors (Dent et al., 2017).

Table 1. Phytochemicals of the methanol extract of *G. diplopetala* leaves

Class of phytochemicals	Test	Observations	Results
Phenolics	Ferric chloride	Green solution	+++
Tannins	Ferric chloride	Blu black precipitate	+++
Flavonoids	Lead acetate	Yellow precipitate	+++
Terpenoids	Salkowski	Reddish brown	+
Saponins	Frothing	Honeycomb froth	+
Steroids	Liebermann-Burchard	A brown ring at the junction of the two layers	+
Alkaloids	Meyer's	-	-
Anthraquinone derivatives	Borntrager's	-	-
Anthraquinones glycosides	Borntrager's	-	-
Aromatic smell	Organoleptic	-	-

[#]+/+++ indicates positive results with increasing intensity or amount of precipitate; - indicates negative result

Evaluation of antioxidant activity, TPC, and TFC

Table 2 shows that the inhibitory concentration (IC_{50}) of ascorbic acid was 6.60 µg/mL, whereas the sample extract was 4.44µg/mL. Interestingly, the IC_{50} of *G. diplopetala* leaf extract is lower than that of ascorbic acid. In accordance with the research by Phongpaichit et al. (2007), extracts with IC_{50} values in the range of 10

to 50 mg/mL are thought to have strong antioxidant activity. Thus, *G. diplopetala's* leaves were proved to possess strong antioxidant activity. The extract contained 32.586 ± 3.044 mgGAE/g TPC as calculated using the equation (Y= 0.0367x + 0.013, R2 = 1) derived from the gallic acid calibration curve. Meanwhile, TFC was 4.461 ± 2.08 mgCE/g as determined based on catechin standard curve (Y= 0.0102x + 0.0345, R2 = 0.09984). This result correlated with the previous study by Kristiani et al. (2018) in which the TFC of the leaves of *G. diplopetala* is lower compared to TPC. Besides, TPC is significantly correlated with TFC and total tannin content (TTC), which indicated that TFC and TTC mainly contributed to the leaves' TPC. Many studies have documented the relationship between the antioxidant activity and phenolic concentration (Alamzeb et al., 2023). Phenolic compounds that contain free hydroxyl groups are linked to strong radical scavenging activities, hence contributing to the extracts' antioxidant activity (Stanković, 2011),

Table 2. Total phenolic content, total flavonoid content and antioxidant activity of the methanol extract of *G. diplopetala* leaves

Sample	**Total phenolic content** (mgGAE/g)	**Total flavonoid content** (mgCE/g)	IC_{50} (µg/mL)
Methanol extract of *G. diplopetala* leaves	32.57 ± 3.04	4.46 ± 2.08	4.44 ± 0.31
Ascorbic acid	nd	nd	6.60 ± 1.63

[#]nd indicates not determined

TLC bioautographic screening of antioxidants

TLC analysis of the methanol extract of *G. diplopetala's* leaves showed the presence of six main phytochemicals, which include the terpenoids labelled as GD1, GD2, GD3, GD4, and the phenolics GD5 and GD6. Among these phytochemicals, GD1, GD5 and GD6 were antioxidant active as screened via TLC bioautographic antioxidant assay (Figure 1 and Table 3). The presence of the terpenoid and phenolic antioxidants explains the findings by Kristiani et al. (2018) for the positive but insignificant correlation between TPC, TFC, TTC and antioxidant activity of the methanol extract.

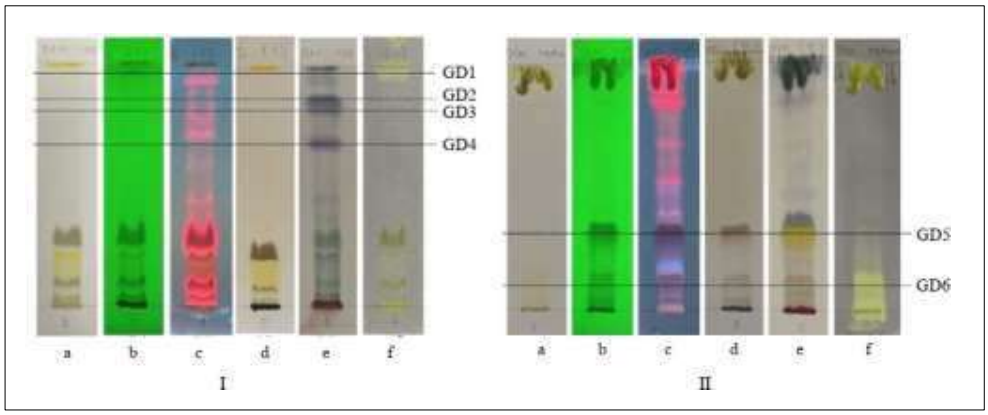

Fig. 1. TLC bioautographic screening of methanol extract of *G. diplopetala* leaves developed by using solvent systems, I, dichloromethane and II, dichloromethane: methanol (9:1) and visualised under a, visible light; b, shortwave UV light (254 nm); c, longwave UV light (366 nm); d, sprayed with $FeCl_3$ solution; e, sprayed with Vanillin/H_2SO_4 with subsequent heating at 110°C and f, sprayed with 0.05.% DPPH in methanol.

Table 3. TLCand antioxidant bioautography profile of the methanol extract of *G. diplopetala* leaves

Solvent system	Labelled compound	Rf x 100	Visible light	UV254	UV366	$FeCl_3$	Vanillin /H_2SO_4	DPPH	Comment
DCM	GD1	98	-	-	-	-	Purple	Pale yellow	Antioxidant
	GD2	86	-	-	-	-	Blue	-	Terpenoid
	GD3	83	-	-	-	-	Purple	-	Terpenoid
	GD4	69	-	-	-	-	Purple	-	Terpenoid
DCM: Methanol (9:1)	GD5	31	-	Black	Dark purple	Dark blue	Yellow	Pale yellow	Phenolic, Antioxidant
	GD6	11	Yellow	Black	Purple	Dark blue	Yellow	Pale yellow	Phenolic, Antioxidant

[#]- indicates no observation of colour

CONCLUSION

This study reveals the promising antioxidant activity of the methanol extract of *G. diplopetala* leaves in the presence of terpenoids and phenolic antioxidants, as well as high total phenolic content. Thus, the findings provide valuable insights regarding the potential therapeutic benefits of *G. diplopetala* leaves.

ACKNOWLEDGEMENTS

The authors would like to thank the Kulliyyah of Pharmacy, International Islamic University Malaysia, for supporting this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alamzeb, M., Khan, B., Ullah, I., Omer, M. & Adnan 2023. Natural antioxidants: An update. In: de Oliveira, M.S., de Aguiar Andrade, E.H., Kumar, R. & Suraj, N.M. (eds.) *Medicinal Plants – Chemicals, Biochemicals, and Pharmacological Approaches*. DOI: 10.5772/intechopen.112462

Bobo-Garcia, G., Davidov-Pardo, G., Arroqui, C., Virseda, P., Marin-Arroyo, M.R., & Navarro, M. 2014. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science of Food and Agriculture*. DOI: 10.1002/jsfa.5633.

Dent, M., Dragović-Uzelac, V., Penić, M., Bosiljkov, T. & Levaj, B. 2013. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (*Salvia officinalis* L.) extracts. *Food Technology and Biotechnology* 51: 84–91.

Herald, T.J., Gadgil, P. & Tilley, M. 2012. High-throughput microplate assay for screening flavonoid content and DPPH-scavenging activity in sorghum bean and flour. *Journal of the Science of Food and Agriculture*. DOI: 10.1002/jsfa.5633

Iqbal, E., Abu Salim, K. & Lim, L.B.L. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University – Science* 27:224-232.

Kristiani, E.B.E, Kasmiyati, S. & Palekahelu, N.Y.C. 2018. Aktivitas antioxidant dan antioxidant dan antibakteri ekstrak daun kapehu (*Guioa diplopetala*). *Prosiding Seminar Nasional Pendidikan Biologi*, pp. 266–272. Salatiga: Program Studi Biologi FB-UKSW

Neha, K., Haider, R., Pathak, A. & Yar, M. S. 2019. Medicinal prospects of antioxidants: A review.

European Journal of Medicinal Chemistry 178. DOI: 10.1016/j.ejmech.2019.06.010 Phongpaichit, S., Nikom, J., Rungjindamai, N., Sakayaroj, J., Hutadilok-Towatana, N., Rukachaisirikul, V. & Kirtikara, K. 2007. Biological activities of extracts from endophytic fungi isolated from *Garcinia* plants. *FEMS Immunology & Medical Microbiology* 51(3): 517–525.

Pratiwi, R.A. & Nurlaeni, Y. 2022. Detection of saponin in underutilized Indonesian Sapindaceae at Cibodas Botanic Garden. *Biogenesis* 10(2): 181-189.

Rabizadeh, F., Mirian, M.S., Doosti, R., Kiani-Anbouhi, R. & Eftekhari, E. 2022. Phytochemical classification of medicinal plants used in the treatment of kidney disease based on traditional Persian medicine. *Evidence-based Complementary Alternative Medicine*, *8022599*. DOI: 10.1155/2022/8022599

Stankovic, M. S. 2011. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science* 33(2011): 63-72.