Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.201

RESEARCH ARTICLE

Open Access

Antioxidant activity of *Crescentia cujete* extract: effects on oxidative stress in the hearts of hypoxic rats

David Limanan*, Brandon Alexander Setiady, Belinda Sentosa, Alfred H Alphanto, Selly Herlia Rudianti, Frans Ferdinal

Department of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia, 11440
*Corresponding author: Jalan Roda No.100/70 Bogor, 16141, Indonesia. Email: davidlimanan@gmail.com

ABSTRACT

Background: The heart is highly susceptible to oxidative stress. Hypoxia can induce oxidative stress in the heart, leading to cardiac damage.

Objective: This study investigated the antioxidant effects of *Crescentia cujete* extract on oxidative stress in hypoxic rat hearts.

Methods: Antioxidant capacity was evaluated using DPPH assay. Sprague Dawley rats were divided into eight groups: four received *C. cujete* extract (400 mg/kg/day for 14 days) and four served as controls. Hypoxia was induced for 3, 7, and 14 days using a hypoxic chamber (8% O_2, 92% N_2). Heart tissue was analyzed for malondialdehyde (MDA), glutathione (GSH), and catalase activity.

Results: *C. cujete* extract demonstrated moderate antioxidant capacity (IC50 = 158.45 µg/mL). In extract-treated rats, MDA levels were significantly lower compared to controls, while catalase activity was significantly higher. GSH levels were higher in treated groups but not statistically significant. Histopathological analysis revealed less cardiac necrosis in extract-treated groups.

Conclusion: *C. cujete* extract demonstrates protective effects against hypoxia-induced cardiac oxidative stress. These findings suggest potential as complementary therapy for oxidative cardiac damage, although further studies are needed to establish clinical efficacy and safety.

Keywords: *Crescentia cujete*, antioxidant, oxidative stress, hypoxia, heart

Introduction

The heart is highly susceptible to oxidative stress, particularly under conditions of chronic hypoxia. Oxidative stress is a state of imbalance between oxidants and antioxidants, leading to tissue damage [1,2]. Oxidants include free radicals and other reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3,4]. Under physiological conditions, ROS and RNS play crucial roles in various cellular processes, including signal transduction, immune defense, and oxygen tension regulation [5]. However, excessive production of ROS and RNS can lead to damage to biomolecules, including carbohydrates, lipids, proteins, and DNA, resulting in protein dysfunction, mutations, and cell membrane damage [6].

Malondialdehyde (MDA) is a biomarker produced as a result of lipid peroxidation and serves as an indicator of oxidative damage. To counteract the effects of ROS and RNS, human cells possess antioxidants, which are stable molecules that can neutralize oxidants before they interact with macromolecules [7,8]. Key endogenous antioxidants include glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) [7,8].

Given the importance of antioxidants in combating oxidative stress, there is growing interest in natural sources of these compounds. Indonesia is rich in natural resources, including plants used in traditional medicine. One such plant is *Crescentia cujete*, which has been documented

for its medicinal properties [9,10,11]. *Crescentia cujete*, native to South and Central America, contains secondary metabolites such as flavonoids, phenolics, and tannins, which exhibit antioxidant properties [12]. This study aims to evaluate the effects of *C. cujete* leaf extract on reducing oxidative stress in the hearts of rats subjected to chronic hypoxia.

Methods

This study was conducted in the Biochemistry and Molecular Biology laboratory at the Faculty of Medicine, Tarumanagara University. Sprague Dawley rats were obtained from the Experimental Animal Laboratory, Puslitbang Biomedis dan Teknologi Dasar Kesehatan, Badan Litbangkes, where the rats were received and force-feeding was conducted. The Brine Shrimp Lethality Test (BSLT) assay was performed at Herbarium Bogoriense, Institut Pertanian Bogor. *Crescentia cujete* plants were obtained from Taman Buah Mekarsari, Cileungsi, Bogor. This study received ethical approval from the Faculty of Medicine Research Ethics Committee at Trisakti University with code number: 125/KER/FK/XII/2017.

Preparation of the ethanol extract of *C. cujete*

Leaves of the *Crescentia cujete* plant were dried and ground into simplicial powder, then extracted using the maceration method with ethanol solvent. The simplicial powder was soaked in absolute ethanol and left at room temperature for 48 hours. The translucent filtrate was collected, and the maceration process was repeated twice. The filtrate was filtered through Whatman filter paper and then evaporated using a Heidolph Rotary Evaporator to remove ethanol.

Procedures on experimental animals and assays

In this experiment, we used male Sprague Dawley rats that were 10-12 weeks old, weighing 180-200g, and healthy. Rats were acclimatized for one week. The rats were divided into eight groups: four groups received the *C. cujete* leaf ethanolic extract, while four groups served as controls without extract treatment.

The control groups (P1-P4) received no extract, while the experimental groups (P5-P8) received *C. cujete* leaf ethanolic extract at a dosage of 400 mg/kg/day for 14 days. Hypoxia was induced using a hypoxic chamber with gas containing 8% oxygen and 92% nitrogen. Groups P1 and P5 were not exposed to hypoxia, while groups P2 and P6, P3 and P7, and P4 and P8 were exposed to hypoxia for 3, 7, and 14 days, respectively.

After 14 days, the rats were removed from the chamber and anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). The chest was cleaned with antiseptic, and an incision was made to remove the rat's heart.

In vitro assays

Phenolic compounds in the extract were detected using the $FeCl_3$ method, flavonoids using the HCl 2N method, alkaloids using the Mayer and Dragendorff methods, and terpenoids and steroids using the Salkowski method. The total antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and toxicity was assessed using the Brine Shrimp Lethality Test (BSLT) method.

In vivo assays

The concentration of MDA in the heart was measured using the Wills E.D. method, GSH using Matt Ellman's method, and catalase activity was measured using the Mates method. Statistical analysis was performed using the Mann-Whitney and Pearson tests with GraphPad Prism v.7.0. Variables were considered significant if $p < 0.05$.

Results

Phytochemical screening results

Results of the qualitative phytochemical screening show that the extract contains phenolics, flavonoids, and terpenoids (Table 1).

Total antioxidant capacity analysis

The antioxidant capacity of the *C. cujete* leaf ethanolic extract was measured using the DPPH

Table 1. Phytochemical screening results of *Crescentia cujete* extract

No.	Compound group	Result	Relevant Information
1.	Flavonoids	+	Red precipitate
2.	Phenolics	+	Green precipitate
3.	Steroids	-	No precipitate or color
4.	Terpenoids	+	Ring with reddish brown color
5.	Alkaloids (Meyer)	+	White precipitate
6.	Alkaloids (Dragendorff)	+	Orange precipitate

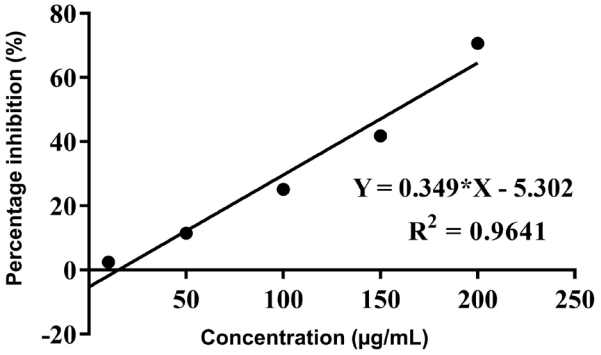

Figure 1. Antioxidant activity of *C. cujete* extract. Graph of percentage inhibition against concentration for the extract.

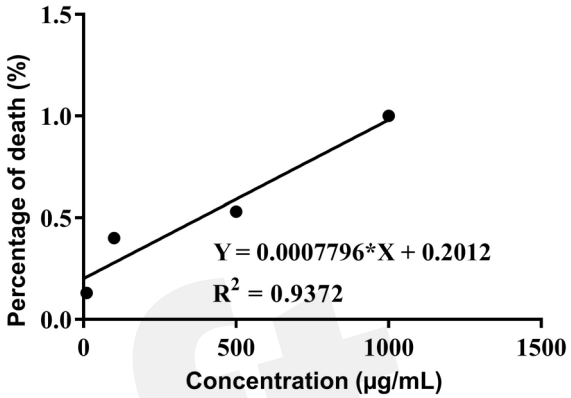

Figure 2. Toxicity assessment of *C. cujete* extract. Graph of the percentage of death against concentration (µg/mL) for the extract.

method. The concentrations used, absorbances measured, percentage inhibition, linear equation determined, and the IC50 can be seen in Figure 1. It was determined that *Crescentia cujete* has moderate antioxidant properties.

Toxicity test results

The toxicity of the extract was measured using the BSLT method, which was repeated three times. The concentrations used, the larval death percentage, and the graph to obtain the linear equation needed to find the LC50 are shown in Figure 2. It was determined that the LC50 was 383.27 µg/mL, indicating moderate toxicity.

MDA measurement results

MDA levels in the heart increased with the duration of hypoxia in both control and extract-treated groups. Statistical analysis showed a significant difference (Mann-Whitney, $p < 0.05$) in MDA levels between the groups that were exposed to hypoxia (3, 7, and 14 days) and the normoxia group. Additionally, MDA levels were higher in the control group, and there was a significant difference (Mann-Whitney, $p < 0.05$) in MDA levels between control and extract-treated groups (Figure 3).

GSH measurement results

GSH levels in the heart decreased with the duration of hypoxia in both extract-treated and control groups. Statistical analysis (Mann-Whitney, $p < 0.05$) showed a significant difference in GSH levels between hypoxia-exposed groups (3, 7, and 14 days) and the normoxia group. While GSH levels were higher in extract-treated groups, the difference between extract-treated and control groups was not statistically significant (Figure 4).

Catalase specific activity assay results

Catalase activity in the heart increased in groups exposed to hypoxia for 3 days but declined with

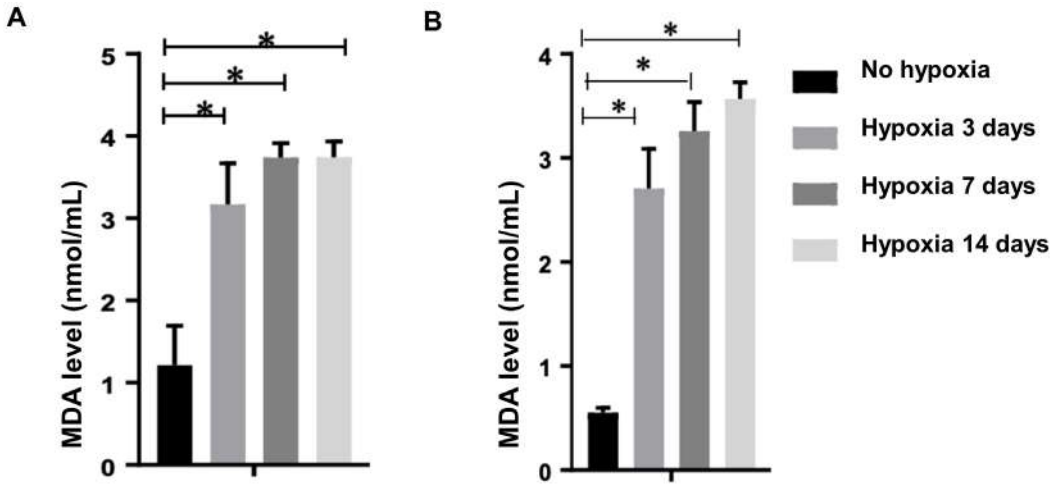

Figure 3. Effect of *C. cujete* extract on cardiac MDA levels under hypoxic conditions. MDA levels in control group (A) and extract-treated group (B).

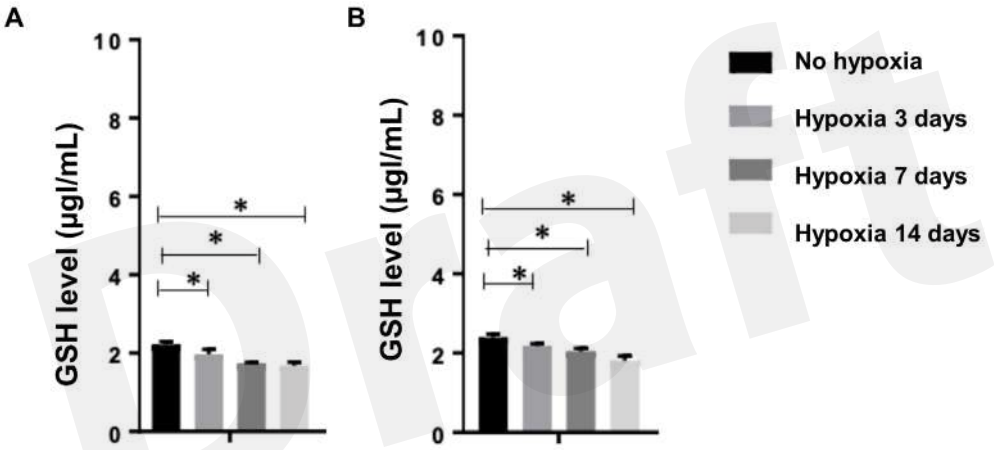

Figure 4. Effect of *C. cujete* extract on cardiac GSH levels under hypoxic conditions. GSH levels in control group (A) and extract-treated group (B).

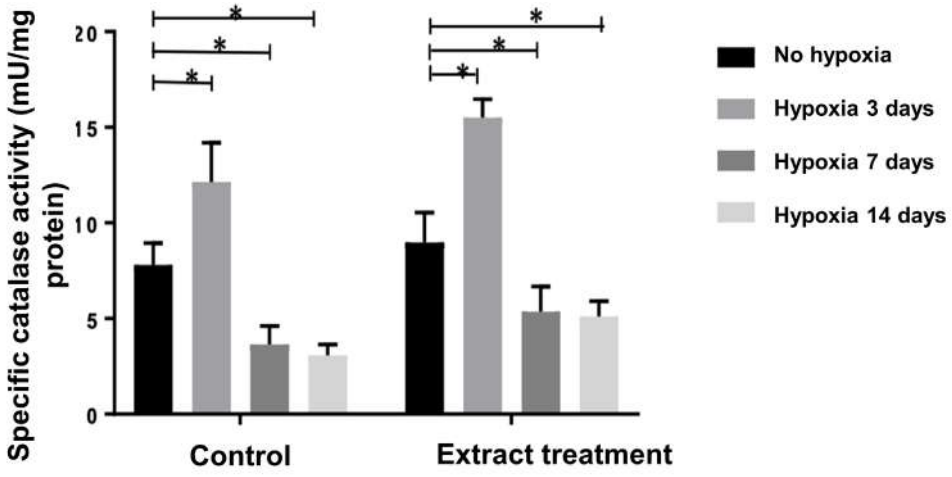

Figure 5. Effect of *C. cujete* extract on cardiac catalase activity under hypoxic conditions. Catalase activity in control groups (left) and extract-treated groups (right).

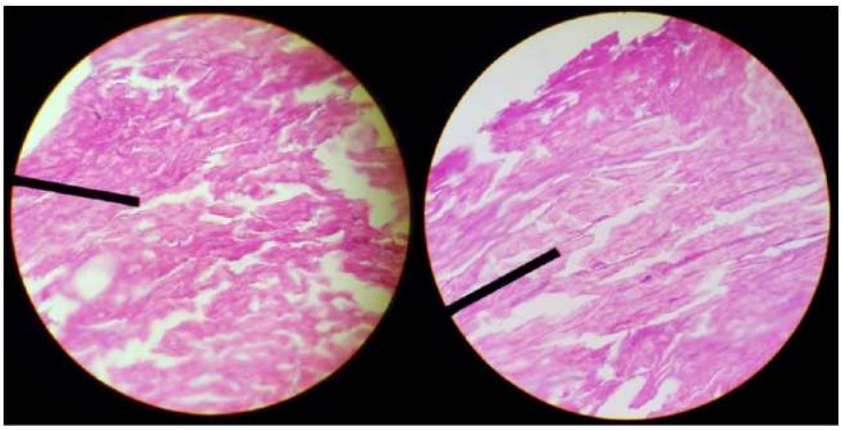

Figure 6. Histopathological analysis of cardiac tissue following chronic hypoxia. Histopathological images of heart tissue after 14 days of hypoxia. Left panel shows the histopathology of rat heart tissue from the control group, while the right panel shows the histopathology of the extract-treated group.

prolonged hypoxia in both extract-treated and control groups. Statistical analysis (Mann-Whitney, $p < 0.05$) revealed a significant difference in catalase activity between hypoxia-exposed groups (3, 7, and 14 days) and the normoxia group. Catalase activity was higher in extract-treated groups, with a significant difference (Mann-Whitney, $p < 0.05$) between extract-treated and control groups (Figure 5).

Histopathology of the hearts after hypoxia

Microscopic examination of the heart tissue from both extract-treated and control groups (Figure 6) revealed the presence of necrosis in both samples. However, more extensive necrosis was present in groups that were not treated with *C. cujete* extract.

Discussion

Phytochemical screening tests revealed that *Crescentia cujete* leaves contained phenolics, flavonoids, terpenoids, and alkaloids. These results are supported by previous research that *C. cujete* contains these compounds [13,14,15]. Studies have demonstrated that phenolic compounds exhibit various pharmacological functions, including antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties. Flavonoids have shown anti-cancer activity, while terpenoids have been shown to have cytotoxic activity towards bacteria and fungi. In addition, alkaloids possess antibacterial, antifungal, antimalarial, anticancer, and antihypertensive effects [16].

The IC_{50} of *C. cujete* leaf extract obtained was 158.45 µg/mL, classifying it as a moderate antioxidant. Das et al. [13] reported that an IC_{50} of 80.21 µg/mL, and Anwuchaepe et al. [17] reported an IC_{50} of 569.22 µg/mL for *C. cujete* leaf extract. The differences in results have been attributed to the different solvents used. The antioxidant capacity of the extract comes from the phenolic and flavonoid compounds, indicating the extract's potential to help mitigate oxidative stress. Although compounds like vitamin C show higher total antioxidant capacity, consumption of vitamin C has been shown to cause nausea, vomiting, headaches, and diarrhea. *C. cujete*, on the other hand, has been shown to treat these side effects.

The result of the Brine Shrimp Lethality Test shows an LC_{50} of 383.27 µg/mL, which categorizes it as having moderate toxicity. This is attributed to the flavonoid compounds, which have antioxidant properties. These results are supported by previous research conducted by Billacura et al. [14], which obtained an LC_{50} of 220 µg/mL.

MDA levels in the heart showed an increase in both extract-treated and control groups, increasing with the duration of hypoxia. This leads to increased oxidant production, which oxidizes polyunsaturated

fatty acids. MDA levels were consistently lower in extract-treated groups than in control groups, as the extract contains various compounds that act as antioxidants, preventing lipid peroxidation. Anwuchaepe et al. [17] found that the hearts of rats given the extract had lower MDA levels compared to those that were not treated.

GSH levels were shown to decrease under conditions of stress (hypoxia), where elevated ROS production causes increased GSH consumption for cellular protection. GSH is oxidized to glutathione disulfide (GSSG) in a reaction catalyzed by glutathione peroxidase, which is important for neutralizing oxidants. This study supports this theory by showing a significant difference in GSH levels between normoxic and hypoxic groups in both extract-treated and control groups. It also shows decreased GSH levels, indicating higher ROS production and that antioxidants are consumed to counteract the ROS, leading to decreased antioxidant levels. This proves that endogenous antioxidants work to neutralize oxidants that are formed during hypoxia.

In groups treated with *C. cujete* ethanolic extract, GSH levels were higher than in control groups, although this difference was not statistically significant. This suggests that supplementation with exogenous antioxidants from the extract may support endogenous antioxidants in counteracting elevated ROS levels.

Catalase-specific activity was highest in normoxic rats and decreased with prolonged hypoxia. A significant difference in catalase activity was observed between hypoxic and normoxic groups. Chronic systemic hypoxia induces increased ROS production, which may overwhelm endogenous antioxidants. Catalase activity initially increased in rats exposed to hypoxia for 3 days, likely due to compensatory upregulation of enzyme expression in response to elevated ROS. However, after 7 and 14 days of hypoxia, catalase-specific activity declined. This may be due to enzyme inhibition and/or inactivation at higher H_2O_2 concentrations [18]. A significant difference in catalase activity between extract-treated and control groups suggests that the extract enhances catalase function in neutralizing ROS.

Histopathological analysis revealed necrosis in the hearts of hypoxia-exposed rats. Hypoxia can deplete ATP, disrupting Na^+/K^+ pumps, leading to abnormal membrane depolarization and Ca^{2+} influx. This activates phospholipases and Ca^{2+}-dependent proteases, triggering necrosis. However, extract-treated groups exhibited less necrosis, indicating that antioxidants from the extract may protect the heart from oxidative stress.

Conclusion

Hypoxic conditions cause elevated ROS production that can damage heart tissue. This is demonstrated by increased MDA levels, decreased GSH levels and catalase activity, along with necrosis of the heart tissue. *C. cujete* may counteract these effects because it possesses various secondary metabolites like phenolics, flavonoids, terpenoids, and alkaloids, along with moderate total antioxidant capacity. This study showed that in rats fed *C. cujete* extract, there was less necrosis, decreased MDA levels, higher GSH levels, and increased catalase activity compared to rats that were not treated with *C. cujete* extract. The moderate toxicity of *C. cujete* observed in the BSLT indicates the need for careful dosage consideration in future therapeutic applications.

Acknowledgment

None.

Funding

None.

Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

Author contributions

DL: Conceptualization, Methodology, Writing – Original Draft. BAS, DL: Data Curation, Formal Analysis, Visualization. BAS, BS, AHA, SHR: Investigation, Resources, Validation. DL, BAS, BS, AHA, SHR, FF: Supervision, Writing – Review & Editing.

Received: December 12, 2024
Revised: May 25, 2024
Accepted: June 20, 2024
Published: June 24, 2025

References

1. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol. 2011;301(6):H2181-H2190. https://doi.org/10.1152/ajpheart.00554.2011
2. Vassalle C, Petrozzi L, Botto N, Andreassi MG, Zucchelli GC. Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. J Intern Med. 2004;256(4):308-315. https://doi.org/10.1111/j.1365-2796.2004.01373.x
3. Clarkson PM, Thompson HS. Antioxidants: what role do they play in physical activity and health? Am J Clin Nutr. 2000;72(2 Suppl):637S-46S. https://doi.org/10.1093/ajcn/72.2.637S
4. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89-96. https://doi.org/10.59566/IJBS.2008.4089
5. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1):47-95. https://doi.org/10.1152/physrev.00018.2001
6. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol. 2011;25(3):287-299. https://doi.org/10.1016/j.bpobgyn.2010.10.016
7. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-126. https://doi.org/10.4103/0973-7847.70902
8. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19. https://doi.org/10.1097/WOX.0b013e3182439613
9. Jolene E. Yukes, Michael J. Balick. Dominican Medicinal Plants : A Guide for Health Care Providers. 2nd ed. The New York Botanical Garden; 2010. 295-298 p.
10. DeFilipps RA, Maina SL, Crepin J. Medicinal Plants of the Guianas (Guyana, Surinam, French Guiana). Department of Botany, National Museum of Natural History, Smithsonian Institution,; 2004. 50-51 p.
11. T.K. L. Edible Medicinal and Non-Medicinal Plants. Vol. 1. Springer Science+Business Media B.V; 2012. 480-485 p. https://doi.org/10.1007/978-90-481-8661-7
12. Das, N., Islam, M.E., Jahan, N. et al. Antioxidant activities of ethanol extracts and fractions of Crescentia cujete leaves and stem bark and the involvement of phenolic compounds. BMC Complement Altern Med 14, 45 (2014). https://doi.org/10.1186/1472-6882-14-45
13. Das N, Islam ME, Jahan N, et al. Antioxidant activities of ethanol extracts and fractions of Crescentia cujete leaves and stem bark and the involvement of phenolic compounds. BMC Complement Altern Med. 2014;14:45. Published 2014 Feb 4. https://doi.org/10.1186/1472-6882-14-45
14. Billacura MP, Pangcoga KKJ. Phytochemical screening, cytotoxicity, mutagenicity, antimutagenicity, and protective potentials of the different solvent extracts from the air-dried leaves of Crescentia cujete Linn. International Journal of ADVANCED AND APPLIED SCIENCES. 2017;4(4):118-126. https://doi.org/10.21833/ijaas.2017.04.017
15. Parente FGG, Oliveira AP de, Rodrigues CMSC, de RG, Almeida JRGS. Phytochemical screening and antioxidant activity of methanolic fraction from the leaves of Crescentia cujete L. (Bignoniaceae). 2016;8(2):231-236.
16. Wink M. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. Medicines (Basel). 2015;2(3):251-286. Published 2015 Sep 8. https://doi.org/10.3390/medicines2030251
17. Anwuchaepe AU, Onyegbule FA, Ajaghaku DL, Nwafor FI, Okoye FBC. Evaluation of the in vivo antioxidant, toxicological and chromatographical profiling of leaf extract and fractions of Crescentia cujete Linn. (Bignoniaceae). Asian Pacific Journal of Health Sciences. 2017;4(3):43-54. https://doi.org/10.21276/apjhs.2017.4.3.8
18. Lardinois, Olivier M, et al. Reversible Inhibition and Irreversible Inactivation of Catalase in Presence of Hydrogen Peroxide. Biochimica et Biophysica Acta (BBA) Protein Structure and Molecular Enzymology, vol. 1295, no. 2, 1996, pp. 222-238. https://doi.org/10.1016/0167-4838(96)00043-X