Original Article

Antioxidant Capacity Variation Across Different Parts of *Allamanda cathartica* (Golden Trumpet) Plant

Dissanayake D. M.W.L[1], Bandara J. M. V.R.J[2], Tennakoon T. M.S.A[1], Kavindhya I.S[1], Sandeepa K.D[1], Ratnayake W.M.K.M [2]

[1]Department of Pharmacy and Pharmaceutical Sciences, Faculty of Health Sciences, CINEC Campus, Sri Lanka

[2]Department of Cosmetic Science, Faculty of Health Sciences, CINEC Campus, Sri Lanka

kalpani.ratnayake@cinec.edu

ABSTRACT

Allamanda cathartica is a herbal plant in the Apocynaceae family, often known as "Wel Rukaththana" in Sinhala, "Thimble Lady," or "Golden Trumpet" in English. Traditional medicine has utilized this plant's leaves, roots, stems, blossoms, and entire plant to cure a variety of illnesses since ancient times. Even though *A. cathartica* is a commonly used herb, the literature review indicated that there is a scarcity of published scientific evidence about its therapeutic usefulness. Hence, the present study aimed to evaluate the comparison of antioxidant activity of fresh leaves, roots, stems and flowers of this pant by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Fresh plant materials were collected from the Colombo district and authenticated. Hot aqueous extracts of fresh leaves (AEFL), roots (AEFR), stems (AEFS) and flowers (AEFF) were prepared with 3 g/mL concentrations. *In-vitro* antioxidant activity of the concentration gradients of each extract was evaluated by using a DPPH assay. Ascorbic acid (6.25 µg/mL- 25 µg/mL) was used as a positive control. The radical scavenging activity of test samples was expressed as an EC_{50}. The hot aqueous extract showed antioxidant activity with an EC_{50} value of 10.92 µg/mL, 22.10µg/mL, 23.76 µg/mL and 27.38 µg/mL for leaves, flowers, roots and stems respectively while the ascorbic acid showed an EC_{50} value of 13.40 µg/mL. In conclusion, the results showed that AFEL has a significant ($p < 0.05$) antioxidant potential than AEFR, AEFS and AEFF. Hence, fresh leaves of *A. cathartica* have been identified as the most potential part for antioxidant activity among tested plant parts.

Index Terms- Allamanda Cathartica, Antioxidant, DPPH

INTRODUCTION

Excessive levels of free radicals can accumulate within the human body cells, and this can lead to certain degenerative diseases such as atherosclerosis, ischemic heart diseases and cancers [1]. These free radicals can alter the structure and function of biomolecules within cells. These alterations have the potential to cause cancer or even mutagenesis in healthy cells [2]. Hence, the human body needs antioxidants to protect the body from overabundance of free radicals.

There are different antioxidants with natural and synthetic origins. While butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) are some examples of synthetic antioxidants, organic acids, phenols, flavonoids, tannins and curcuminoids are some examples of naturally occurring antioxidants in plant sources [3]. The natural antioxidants can be dispersed throughout the plants including leaves, stems, roots, bark, flowers, fruits and seeds. As some synthetic antioxidants such as BHA and BHT have reported certain adverse effects such as liver damage and carcinogenic potential [4], considering the comparatively less toxic natural antioxidants have a huge demand. Hence, recent research has paid attention to identifying the antioxidant potential of herbal plants and the isolation of antioxidant compounds from them.

Allamanda cathartica (named “Wel Rukaththana” in Sinhala; “Golden Trumpet” in English) is a herbal plant that belongs to the family Apocynaceae. This is commonly found as an ornamental shrub in the garden (Plate 1) in tropical and subtropical regions [5]. This is a perennial plant and mainly grows in Sri Lanka as an ornamental plant in home gardens and rode sides in all wet, dry

and intermediate zones. The leaves, roots, stems, flowers and the entire plant have been used for centuries in traditional medicine to treat various diseases [6, 7]. Hence, *Allamanda cathartica* plays an important role in Ayurveda and Unani medicine. An infusion of the bark and leaves of this plant is used as a purgative, in traditional medicine [6]. Also, on the bites of insects, the paste of root is administered. Further, the plant is used to treat liver tumours, jaundice, splenomegaly, malaria, and severe stomach discomfort. The plant's compounds aid in reducing inflammation and enhancing blood circulation [7].

In addition to the traditional uses, the scientific literature also showed that different parts of this plant contain numerous pharmacological activities such as anti-inflammatory, antioxidant, antidermatophytic, antimicrobial, wound healing, hepatoprotective and antifertility activities [6].

Plate 1: Morphology of *A. cathartica plant*

According to Shivananda *et al*. [8], the aqueous extract of *A. cathartica* showed wound healing activity on excision and incision wound models in Sprague Dawley rats [8]. As shown by Nahar *et al*. [9] dichloromethane extract of the whole plant of *A. cathartica* showed an antidermatophytic effect on two pathogenic dermatophytes *Microsporum gypseum* and *Trichophyton rubrum*. However, they have shown the absence of this activity in the methanolic extract of whole plants [9].

Further, aqueous leaf extract of *A. cathartica* showed reversible suppression of fertility in male Parkes strain mice, when orally administered in the dose of 150 mg/kg of body weight [10]. Pothan and Harindran [11] evaluated the hepatoprotective activity of aqueous and methanol extracts of *A. cathartica* flowers and roots, using the MTT assay. The results showed that 1000 µg/mL of root extract contains 86% protection while flowers contain 81% protection for the same concentration [11].

In 2018, Safitri and his coworkers screened the antioxidant effect of different extracts i.e. aqueous, ethanol and n-hexane, of *A. cathartica* leaves obtained from South Sulawesi [12]. They identified, the aqueous extract of *A. cathartica* (IC_{50}= 44.9 µg/mL) as the strongest antioxidant extract compared to the ethanol and n-hexane extract which showed 106.4 µg/mL and 164 µg/mL respectively as their IC_{50} value. The quercetin which is the reference standard showed 9 µg/mL as its IC_{50} value in DPPH assay [12].

The phytochemical screening of methanol and aqueous extracts of flowers and roots of *A. cathartica* have shown that all extracts contain alkaloids, flavonoids, glycosides, amino acids and starch [11]. Further, there was the absence of anthraquinones, gums and mucilage. However, the methanol extract of roots shows the absence of saponins, although all other tested extracts contained it [11]. However, external environmental variables such as light, temperature, soil water, soil fertility and salinity can have a substantial impact on various plant processes including the synthesis of secondary metabolites, which can ultimately alter the overall phytochemical composition of the plant. Hence, although it is the same plant species, we can see variations in their phytochemistry and medicinal properties depending on geographical variations.

Although different parts of this plant are used in the traditional medicinal system in Sri Lanka, there is a paucity of literature about the biological activities of this plant. Hence, the present study was focused on investigating the antioxidant potential of different parts of *A. cathartica* i.e. leaves, roots, stems and flowers, grown in Sri Lanka. This finding will provide scientific evidence to use aqueous extract of this plant in the development of pharmaceutical and cosmeceutical antioxidant products in future.

MATERIALS AND METHODS

Plant materials

Fresh samples of leaves, flowers, roots and stem of *A. cathartica* were collected from the Colombo district, Sri Lanka. The plant materials of *A. cathartica* were authenticated by the National Herbarium, Department of National Botanical Garden, Peradeniya, Sri Lanka.

Chemicals

The special chemicals of 2-2-diphenyl-1-picrylhydrazyl (DPPH) and Ascorbic acid purchased from Sigma-Aldrich Company, were used for the assays. All other chemicals were analytical grade.

Preparation of the plant extracts

The collected mature fresh parts of *A. cathartica* i.e., leaves, flowers, roots and stems were washed from tap water and then distilled water. After that, all plant materials are cut into small pieces. To prepare the aqueous extract of fresh leaves of *A. cathartica* (AEL), 50 g of small pieces of fresh leaves were refluxed with 150 mL of distilled water for 30 minutes. The extract was filtered using suction filtration, and the filtrate was collected. Same method was followed to prepare an aqueous extract of fresh flowers, roots and stems of *A. cathartica* and they were named AEF, AER and AES respectively.

Evaluation of *in-vitro* antioxidant activity by DPPH assay

According to Ratnayake *et al*. [13], the scavenging activity of the stable 1,1-diphenil-2-picrilhidrazil (DPPH) free radical was used to evaluate the *in-vitro* antioxidant activity [13]. To prepare the 0.25 mM DPPH solution, 10 mg of DPPH was dissolved into 250 mL of absolute methyl alcohol, which was homogenized and transferred to an amber flask, duly labelled. Concentration gradients for each part of *A. cathartica* were prepared by using a stock solution containing a concentration of 1 g/3 mL solution. The Ascorbic acid stock solution 10 mg/mL was prepared by dissolving 0.5 g of Ascorbic acid in 50 mL of distilled water. A series of test solutions of Ascorbic acid with varying concentrations (0.625 µg/mL – 25 µg/mL) were prepared.

The reaction mixtures were prepared by mixing an aliquot of DPPH solution and methanol (in negative control) or test samples (AEL or AEF AER or AES) or Ascorbic acid (in positive control) as shown in Table 1.

After that, the reaction mixtures were allowed to reach a steady state in the dark at room temperature. The absorbances were measured at 517 nm after 30 minutes.

Table 1: Content of reaction mixtures in *in-vitro* antioxidant evaluation by DPPH assay

	NC (mL)	PC (mL)	TS (mL)	CBT (mL)
DPPH solution	1.5	1.5	1.5	NA
Methanol	1.5	NA	NA	1.5
AEL/AEF/ AER/AES Solutions	NA	NA	1.5	1.5
Ascorbic acid solution	NA	1.5	NA	NA

NC: Negative control; TS: Test sample (AEL/AEF/AER/AES); CBT: Colour blank for test sample, PC: Positive control (Standard solution).

All the tests were performed in triplicate for each concentration. Antioxidant activity was measured in terms of radical scavenging activity and the percentage scavenging effect was calculated using the following formula.

$$\text{Scavenging activity (\%)} = \frac{[A_o - A_T]}{A_o} \times 100$$

Where A_0 is the absorbance of the negative control and AT is the absorbance of the test sample (AEL/AEF/AER/AES or Ascorbic acid). The radical scavenging activity of test samples was expressed as a mean of EC_{50} (µg/mL), which is defined as the mean concentration of the antioxidant required to lower the initial DPPH concentration by 50% in each experiment. It was determined by using the graph plotted with the mean concentration of triplicates of each test.

STATISTICAL ANALYSIS

All the results were subjected to descriptive statistics and expressed as mean ± standard deviation (SD). Data were analyzed by using SPSS statistic software. p-values < 0.05 were considered as statistically significant.

RESULTS

DPPH percentage inhibition of the scavenging activity of AEL, AEF, AER and AES are shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively. Also extracts showed significant ($p<0.05$) dose dependent DPPH scavenging activity.

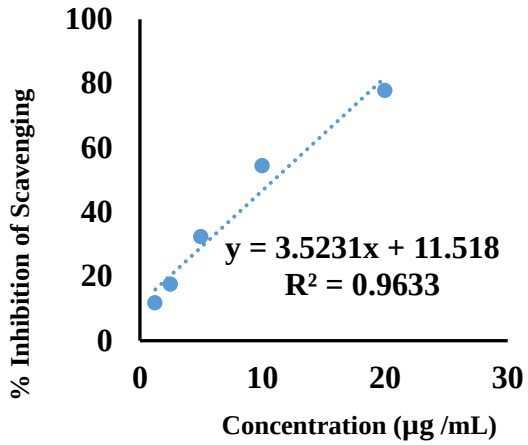

Figure 1–Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *A. cathartica* leave

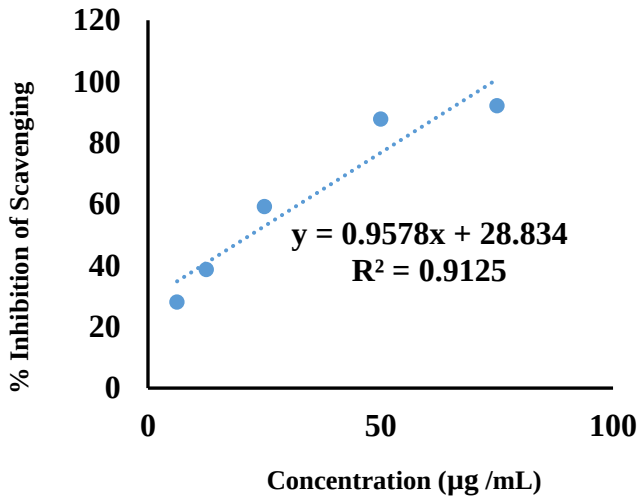

Figure 2–Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *A. cathartica* flowers

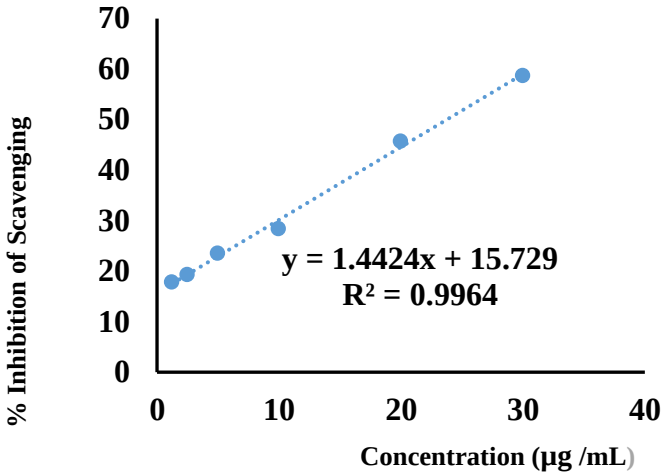

Figure 3–Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *A. cathartica* roots

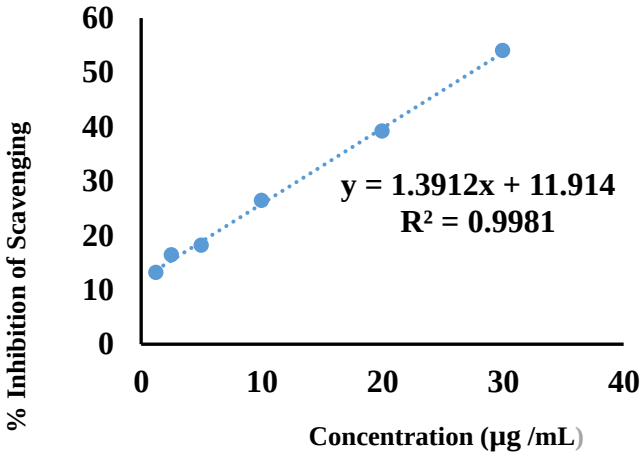

Figure 4–Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *A. cathartica* stem

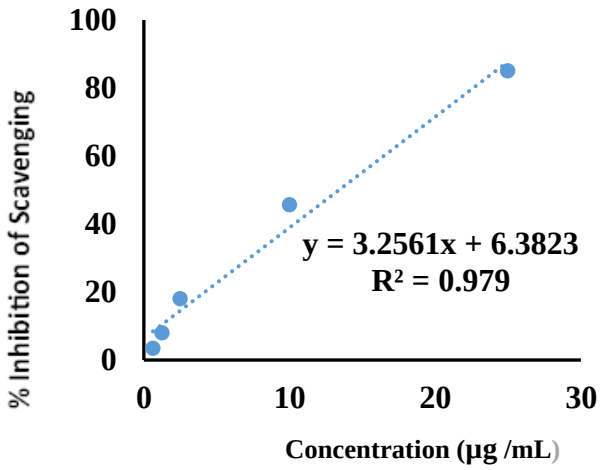

Figure 5 – Standard Curve for Ascorbic Acid in DPPH assay

The results (Table 2) showed that leave extract has more significant antioxidant activity compared to the other three extracts which are prepared from flowers, stems and roots. This activity is higher than standard ascorbic acid, which has an EC_{50} value of 13.40 µg/mL.

Table 2: EC_{50} µg/mL values in DPPH assay

Sample	EC_{50} value
Aqueous extract of *A. cathartica* leaves (AEL)	10.92 µg/mL
Aqueous extract of *A. cathartica* flowers (AEF)	22.10 µg/mL
Aqueous extract of *A. cathartica* roots (AER)	23.76 µg/mL
Aqueous extract of *A. cathartica* stems (AES)	27.38 µg/mL
Ascorbic acid (Positive control)	13.40 µg/mL

DISCUSSION

Because of their safeness, tolerability and non-toxicity, natural antioxidants are considered comparatively superior to synthetic ones [12]. Hence, the free radical scavenging antioxidants of plant origin are abundantly used in the form of vitamins, minerals and nutraceuticals. [13].

Hameeda and coworkers [15] conducted a comparison of the antioxidant activities of roots, shoots, leaves and flowers of *A. cathartica*. According to them, the roots of *A. cathartica*, showed the highest enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Also, it showed the highest total phenolic contents compared to the other test parts of the plant [14]. according to Coelho *et al.* [17], the ethanol extract of dried leaves of *A. cathartica* showed a greater amount of phenolic compounds compared to its flower extract. According to the analyzed standards, they have identified the presence of several phenolic compounds, such as chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid in leaves [15]

Khatoon and his coworkers [1] worked on methanol extracts of leaves and stems of *A. cathartica* prepared by maceration technique. They assessed total phenolics content as mg of gallic acid equivalents (GAE) and the total flavonoid content as mg of quercetin equivalents (QE) The study showed the leaf extract contains total phenolic in 53.35 ± 1.87 mg/GAE/g and total flavonoids in 170.30 ± 0.10 mg QE/g whereas, it was 38.78 ± 0.00 mg/GAE/g and 140.30 ± 0.10 mg QE/g for stem extract respectively [1]. As total flavonoids and phenols are two of the compounds mainly involved in the antioxidant potential of plants, this evidence provides scientific support for the results we received in this study, where it showed leave extract contains more antioxidant activity than the stem in the DPPH assay.

In 2018, Safitri and his co-workers [11] showed that an aqueous extract of *A. cathartica* leaves obtained from South Sulawesi has antioxidant potential with IC_{50}= 44.9 µg/mL. However, our study showed that the EC_{50} value was 10.92 µg/mL for the *A. cathartica* leaves collected from Colombo, Sri Lanka. In addition, Safitri, Handayani and Waris used dried leaves while we used fresh leaves. Hence all of these factors may contribute to the different EC_{50} values shed on the aqueous extract of *A. cathartica* leaves.

CONCLUSION

The fresh hot aqueous extract showed antioxidant activity with an EC_{50} value of 10.92 µg/mL, 22.10µg/mL, 23.76 µg/mL and 27.38 µg/mL for leaves, flowers, roots and stems respectively. The ascorbic acid showed an EC_{50} value of 13.40 µg/mL. In conclusion, the present findings provided scientific evidence for the *in-vitro* antioxidant properties of the different fresh parts of *A. cathartica*. Hence, the leaves of *A. cathartica* displayed comparatively higher antioxidant potential than in other parts. The plant, particularly its leaves, can be used to make pharmaceutical herbal medicines and may be useful in the identification, purification, and isolation of novel phytoconstituents with therapeutic applications.

ACKNOWLEDGEMENT

Authors would like to express gratitude to the Faculty of Health Sciences, CINEC Campus Sri Lanka.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

REFERENCES

1. Khatoon, M., Islam, E., Islam, R., Rahman, A. A., Khurshid Alam, A. H. M., Khondkar, P., Rashid, M.and Parvin, S. 2013. Estimation of total phenol and *in-vitro* antioxidant activity of Albizia procera leaves, BMC Research, 6: 121.

2. Harliansyah, 2001 'MengunyahHaliaMen yahPenyakit', Indonesian Student Associat ion in Malaysia, Artikel.

3. Blainski, A., Lopes, G. C. and de Mello, J. C. P. 2013. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L, Molecules, 18: 6852-6865.

4. Zhu, K., Lian, C, Guo, X, Peng, W., Zhou, H. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ, Food Chemistry, 126: 1122-1126.

5. Loss, A., Teixeira, M. B., Assuncao, G. M., Haim, P. G., Loureiro, D. C., Souza, J. R., 2008. Revista de Ciencias Agrarias, 3: 313-6.

6. Chaithra, A. B., Satish S., Karunakara H. 2016. Therapeutic Uses of *Allamanda Cathartica* Linn. With A Note on Its Phrmacological Actions: a Review, International Journal of Pharma And Chemical Research, 2(4): 227-232.

7. Suprapta, D. N., Khalimi, K. 2012. Anti-fungal activities of selected tropical plants from Bali Island, Phytopharmacology, 2(2): 265-270.

8. Shivananda, N., Poorna, N., Steve, S., Vidyasagar, B., Andrew A. 2006. Evaluation of wound healing activity of *Allamanda cathartica*. L. and *Laurusnobilis.* L. extracts on rats. BMC Complementary and Alternative Medicine, 6 (12): 1-6.

9. Nahar, A., Ashrafuzzaman, S., Islam, M. N., Alam, M. S. 2010. Studies on anti-dermatophytic effect of *Allamanda cathertica*, A Journal of the Bangladesh Pharmacological Society, 5: 5-7.

10. Singh, A., Singh, S. K. 2008. Reversible antifertility effect of aqueous leaf extract of *Allamanda cathartica* L. in male laboratory mice, Andrologia: 40(6): 337-45.

11. Pothan, N., Harindran, J. 2014. *In-vitro* hepatoprotective activity of *Allamanda cathartica linn* on the BRL3A cell lines, International Journal of Institutional Pharmacy and Life Sciences, 4(3): 1-11.

12. Safitri, I. Handayani, V., Waris, R. 2018. Identification and antioxidant activity of Alamanda leaf (*Allamanda chatartica* L) based on solvent variation, International Journal of Advances in Science Engineering and Technology: 5-10.

13. Ratnayake, W. M. K. M., Suresh T. S., Abeysekera, A. M., Salim, N., Chandrika U. G. 2019. Acute anti-inflammatory and anti-nociceptive activities of crude extracts, alkaloid fraction and evolitrine from *Acronychia pedunculata* leaves. Journal of ethnopharmacology. 238:111827.

14. Kumar S. 2011. Free radicals and antioxidants: human and food system. Advanced in Applied Science Research, 2 (1):129–135.

15. Patil, S., Kadam, V., Ghosh, R. 2009. *In-vitro* antioxidant activity of methanolic extract of stem bark of *Gmelina arborea*

Roxb. (Verbenaceae). International Journal of Pharm Tech Research. 1(4):1480–1484.

16. Hameeda, A., Nawazb, G., Gulzarb, T. 2014. Chemical composition, antioxidant activities and protein profiling of different parts of *Allamanda cathartica*, Natural Product Research: 2066-2071.

17. Coelho, H. A., Silveira, L. H. C., Araujo, J. H. B., Kwiatkowski, A. 2016. Antioxidant and antimicrobial potential of phenolic compounds extracted from leaves and flowers of Allamanda cathartica L., Revista Brasileira de Plantas Medicine, 18(3): 650-656.