

INFLUENCE OF LUNAR CYCLES ON ANTHOCYANIN CONTENT OF *JUSTICIA WYNAADENSIS* (NEES) HEYNE EX T. AND.

M.VASUNDHARA*., R. PRIYANKA, , M. NETHRAVATHI AND MANJUNATH SWAMY

Department of Horticulture, UAS(B), GKVK, Bangalore - 560 065

e-mail: vasundhara.vasu@gmail.com

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*Corresponding author

ABSTRACT

Anthocyanins are the most abundant naturally occurring flavonoid pigments and a group of antioxidants known for their therapeutic use. In humans and other mammals, an internal mechanism or the biological clock, imposes circadian rhythms on physiological, biochemical and molecular events. While plants, are unique, their control mechanism is external, specifically the environment. Light has shown to be the most important environmental factors influencing anthocyanin biosynthesis in plants. The present study was established to understand the effect of lunar cycles on the anthocyanin pigment of *Justicia wynaadensis* under Bangalore condition. The results clearly demonstrated the effect of Full moon and new moon days on the accumulation of pigment. The results reported that the leaves collected on New moon day (2nd August, 2016) recorded highest accumulation of anthocyanin pigment of 11.31mg/L. The content gradually decreased from 11.31 mg/L to 0.488 mg/L, while, on Full moon day, the content reduced from 7.3mg/L to 1.428mg/L. The lunar cycles, thus play a predominant role in the accumulation of the pigment. *Justicia wynaadensis* can be considered as a natural antioxidant and as a food supplement in prevention diseases and to stay healthy throughout the year.

INTRODUCTION

A plant is a sessile organism that adapts to its environment through changes in its chemistry. Ayurvedic classics have advocated to collect the medicinal plants according to part used and seasons in order to get desired pharmacological action and therapeutic benefits. For development of high-quality plant based medicines, research in plant rhythms should be attempted to understand the forces acting on the plant and the mechanisms that change their phytochemistry (Cole and Balick, 2010). Across the globe, farmers, craftsmen and healers have carefully observed the phases of the moon, especially while planting, harvesting or collecting plants. Whenever plant materials were required to be strong and resistant to the environment, they were harvested during a waning moon, and medicines were often harvested following the new moon during a waxing period. Swagata *et al.*, 2016, Swagata *et al.*, 2015 have reported the influence of full moon day on the growth and Phyto-constituents of the root, in comparison to new moon day in Ashwagandha plant. The present study embodies on the effect of lunar cycles on the accumulation of anthocyanin content on one of the seasonal shrub of Western Ghats. *Justicia wynaadensis*, an endemic plant of Western Ghats is a seasonal shrub of Acanthaceae family. It is consumed by the local people of Kodagu (Karnataka) only during the Kataka or Adi month of the Hindu calendar (July to August) (Vishma *et al.*, 2017, Vandana and Shanthi, 2017). Only during this period a layer of purple pigment is found along upper and lower epidermis of the leaf. And in the middle cortex of internodal region of the stem the cells are filled with chlorophyll pigment. It is believed that the plant acquires one medicinal property per day and reaches maximum on the 17th day (first week of August) with a total of

eighteen medicinal properties, hence the name Maddu thoppe meaning, Medicinal leaves. The deep bluish purple colored juice (unique flavour) extracted is consumed by local population of Kodagu district only during the monsoon season. This traditional practice is believed to keep the people healthy throughout the year (Greeshma and Shridhar, 2016, Sudha *et al.*, 2011, Patil *et al.*, 2015,). The plant is known to possess anti-proliferative, antibacterial, antioxidant and Anti-inflammatory activities. Also known for its cysteine protease inhibition activity, lowering of cellular cholesterol level and exhibit catalase and peroxidase activities. Ethnomedically, the plant is used as a immune booster, treat asthma and rheumatic swelling. The ethnobotanical reports and comprehensive chemo-profiling is essential to observe and understand the best time to plant and harvest for healthy crops rich in medicinal constituents. The present study was thus planned to understand the effect of lunar cycles on the accumulation of anthocyanin in *J.wynaadensis* under Bangalore conditions.

MATERIALS AND METHODS

Sample Collection

The fresh leaves of *Justicia wynaadensis* were collected from Sugandha Vana and Sanjeevani Vatika, Medicinal and Aromatic plant section, Department of Horticulture, UAS (B), GKVK, Bangalore in the month of August, 2016. The samples were collected during Pournima (Full moon) and Amavasya (New moon) days from August 2016 to December 2016.

Extraction of Anthocyanins

About 50g of the fresh leaves harvested at regular intervals (15days) was respectively weighed and boiled in 500ml of distilled water. The leaves were boiled for 45min until deep

blue purple colour extract is obtained. The extract obtained was filtered using whattman filter paper and refrigerated. The undiluted filtrate was used for quantification of anthocyanins (Vandana and Shanthi, 2017, Sherpa *et al.*, 2014, Tuti *et al.*, 2015).

Total Anthocyanin Content Measurement using pH Differential method

The total anthocyanin content was determined according to spectrophotometric pH-differential method. 1ml aliquot of the extract filtered was mixed with 0.025M potassium chloride buffer (pH 1.0,4ml) and 0.4 M sodium acetate buffer (pH 4.5, 4ml) respectively. The absorbance of the mixture was measured at 510nm and 700nm respectively using UV-Vis spectrophotometer. Absorbance was calculated as $A = [(A_{510} - A_{700}) \text{ at pH } 1.0] - [(A_{510} - A_{700}) \text{ at pH } 4.5]$ with a molecular extinction coefficient of 29,600 for anthocyanin (Vasundhara *et al.*, 2017, Liu *et al.* 2012). The total anthocyanin was calculated as cyaniding-3-glucoside equivalents as per the following equation:

$$\text{Anthocyanin(mg/L)} = (A \times MW \times DF \times 103) / (\epsilon \times L)$$

A- Absorbance ; MW- Molecular weight of cyanidin-3-glucoside(445.0 Da); DF- Dilution factor; V is the final volume (ml); L- cell path length(1cm);m- weight of the dried powder taken(g).

RESULTS AND DISCUSSION

Natural dyes have become a viable alternative in the food industry because of their abundance, simple extraction techniques, non-toxic and biodegradation. Anthocyanins are the most abundant naturally occurring flavonoid pigments which often give a bright red, blue or violet color to plant parts. They are also a group of plant antioxidants known for their therapeutic use. (Yusoff *et al.*, 2014, Stutte and Edney, 2009, Corrêa and Antônio, 2011). In the present study, the anthocyanin content of *J. wynaadensis* leaves collected from Bangalore region on pournima (Full moon) and Amavasya (New moon) days was evaluated. The results reported that the leaves collected on New moon day (2nd August, 2016) recorded highest accumulation of anthocyanin pigment (11.31mg/L) as compared to samples collected on other new moon and Full moon days (Table-1, Fig-1). The anthocyanin content of *Justicia* leaves collected on New moon day gradually decreased from 11.31 mg/L to 0.488 mg/L, while, on Full

Table 1: Anthocyanin content (mg/L) of *Justicia wynaadensis* leaves

Sl.no	Sample collected on	Lunar cycle	Mean Anthocyanin content (mg/L)
1	2/8/16	New moon day	11.32 ± 0.00
2	3/8/16	Regular day	3.92 ± 0.01
3	10/8/16	Regular day	4.20 ± 0.01
4	17/8/16	Full moon day	7.25 ± 0.05
5	1/9/16	New moon day	5.67 ± 0.00
6	16/9/16	Full moon day	5.82 ± 0.01
7	30/9/16	New moon day	2.10 ± 0.00
8	16/10/16	Full moon day	0.61 ± 0.01
9	30/10/16	New moon day	0.83 ± 0.01
10	14/11/16	Full moon day	1.43 ± 0.01
11	29/11/16	New moon day	0.49 ± 0.00

Note: The values represented as Mean ± SEM, are a mean of two replications

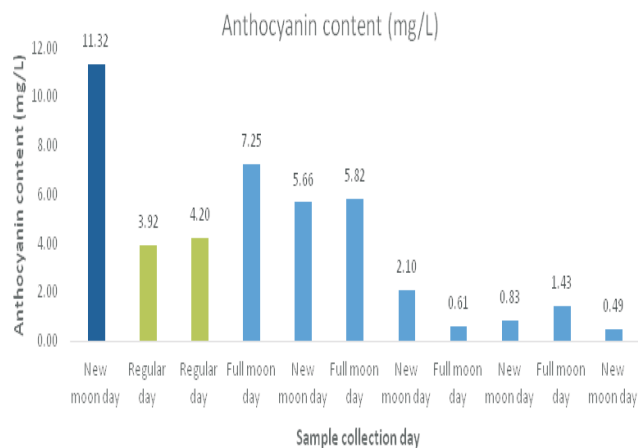


Figure1: Influence of lunar cycles on anthocyanin content (mg/L) of *Justicia wynaadensis*

moon day, the content reduced from 7.3mg/L to 1.428mg/L. The results are in accordance with the literature available (Patil *et al.*, 2015 and Greeshma and Shridhar, 2016). As per the available literature, *J. wynaadensis* of Kodagu region is rich in medicinal value only during the Kataka or Adi month of the Hindu calendar (1st week of August) and similar results were observed even under Bangalore condition. These observations imply that the lunar effect plays a predominant role in accumulation of anthocyanin as compared to the geographical location. Similar studies have been reported in Ashwagandha plant, wherein, the full moon day influences the growth and Phyto-constituents of the root, in comparison to new moon day (Swagata *et al.*, 2016, Swagata *et al.*, 2015).

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