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Extraction of crocin from the flowers of night jasmine (*Nyctanthes arbortristis* Linn.)

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Abstract
The determination of crocin was investigated using conventional method from the flowers of *Nyctanthes arbortristis* Linn. Crocin – a apocarotenoid glycoside is present in the flowers of *N. arbortristis.* This is similarly present in the stigma of saffron. Saffron is conferred as World's most expensive spice, hence is called as a luxury spice. However, the process of procuring the stigma of saffron flowers is indeed a challenging task for the farmers and for research purpose. It is usually adulterated with safflower stigmas coloured with synthetic dye to retain its colour. Therefore, our study would pave way for the extraction of crocin from the flowers of *N. arbortristis*. Based on our findings this study would pave way to the conclusion that the flowers of *Nyctanthes arbortristis* Linn. would be good source in food and pharmaceutical industries.

Keywords: *Crocin, Nyctanthes arbortristis, apocarotenoids*

1. Introduction
Nyctanthes arbor-tristis (Night-flowering Jasmine) belongs to the family Oleaceae, native to southern Asia, from northern India. It is a shrub or a small tree growing to 10 m tall, with flaky grey bark. The leaves are opposite, simple, 6-12 cm long and 2-6.5 cm broad, with an entire margin and have good medicinal properties. The flowers are fragrant, with a five-toeight-lobed white corolla tube with an orange-red centre; they are produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn. The seeds, flowers and leaves possess immunostimulant, hepatoprotective, ant leishmanial, antiviral and antifungal activities (Puri *et al.*, 1994) [1]. Chemical constituents of the flowers contain essential oils, Glucose, Nyctanthin, d-mannitol, Tannin, Glycosides like β-monogentiobioside ester of α crocetin (or crocin-3), β-monogentiobioside, β-digentiobioside ester of α-crocetin (or crocin-1), β-D monoglucoside ester of αcrocetin and carotenoids. The flowers can be used as a source of yellow dye for clothing. A process for the preparation of a bioactive composition from Nyctanthes arbor-trisits having 8-12% of crocin, 20-28% of crocin-2 and 40-55% crocin-3 which comprise of: extracting flowers or its defatted flowers of Nyctanthes arbor-tristis with a polar solvents has been reported by (Gupta *et al.*, 2003) [4]. In preliminary investigations, the orange-red coloured dye obtained from the cold extraction of the tubular calyx indicated that the thin layer chromatography (TLC) profile of the dye resembled that of the dye from saffron. It was reported to be a substitute for the colouring matter of saffron.
Further investigation on calyx indicated the presence of a yellow-orange colour pigment present in N. arbor-tristis which was found identical to crocin, an apocarotenoid called as colouring principle present both in saffron and in the calyx of N. arbor-tristis (Gadgoli *et al.*, 2010) [5]. Spectroscopic analysis confirmed that it is a carotenoid glycoside resembling crocin and the spectroscopic analysis confirmed the structure of aglycone to be crocetin.
Carbohydrates are naturally present in the flowers in a concentration of 15% (w/w) consisting mainly of glucose and gentiobiose. Crocetin, is a carotenoid characterized by two carboxyl groups at the end of a chain of twenty carbon atoms with seven double bonds and four methyl groups and its glycosylated esters, the crocins, responsible for the colouring power, of bright yellow, soluble in water; these last compounds are rapidly hydrolysed to crocetin, insoluble in water and having red colour The yellow colour imparted to foods and beverages is due to the presence of carotenoids and in particular to crocetin and its esters.
The flowers blossom at night and wither the next morning. The methanolic extract of the flowers were also found to have analgesic, anti-inflammatory and antioxidant activity in preliminary studies.

The flowers perish within 20 min and are literally wasted due to their short life. Several biological activities are attributed to this compound. It is usually isolated from Crocus sativus which is highly expensive and not easily available. Isolation of crocin from N. arbor-tristis flowers will be of great significance. Therefore, it was thought worthwhile to investigate if the orange coloured tubular calyx of *N. arbor-tristis* has the resemblance with saffron stigma in the aroma constituents also. Such investigations will bring an economical substitute for saffron from the flowers that are wasted due to short life and these can be utilized effectively. Flowers can be used as a source of yellow colour dye for clothing. It was reported that this colouring compound was present in corolla tubes of flowers of *N. arbortristis* by (Dhingra *et al.*, 1976) [7].

1.1. Types of Carotenoids

Carotenoids can be divided into three main categories, mainly based on the presence or absence of oxygen (lutein, violaxanthin, zeaxanthin, and α cryptoxanthin), chemical structure, and others (apocarotenoids, homocarotenoids, secocarotenoids, norcarotenoids) Currently, there are about 600 identified carotenoids (lipid soluble tetraterpenoids). Xanthophyll's (zeaxanthin, zeaxanthin, violaxanthin, and siphonoxanthin) is the term assigned to carotenoids which contain oxygen and are separated from carotenes based on their polarity and are synthesized in the plastids. Also, their synthesis does not require sunlight, hence, are predominant in light-starved plants (young and etiolated leaves). Nevertheless, carotenoids free of oxygen are called carotenes (lycopene α-carotene, β-carotene) and are exclusively hydrocarbon. The orange hue pigments are vital for photosynthesis; hence, light is involved in the synthesis of carotene. Structurally, carotenoids differ based on functional groups, i.e., hydroxyl and epoxy, and are called carotenols and epoxy carotenoids, respectively. Apocarotenoids, norcarotenoids, homocarotenoids, and secocarotenoids are terms used to describe specific carotenoids produced by an organism which differs based on the number of carbon atoms. Enzymatic and chemical (non enzymatic) oxidative cleavage of carotenoids produces unique biologically important carotenoid derivatives called apocarotenoids (Adadi P *et al.*, 2018) [8].

2. Materials and Methods

2.1. Plant material collection

Flowers of *N. arbortristis* (2-3 kg) were collected from Gokulam colony, Coimbatore district at latitude 11.0197° and the longitude of N, 76.9247° E. It was collected during the month of June to August, 2021. They were collected fresh and subjected to shade drying technique. After the complete moisture is left, they were powdered coarsely using the pulveriser. They were weighed, labelled and stored at 4 °C in sealed in zip lock covers and subjected to extraction.

2.2. Chemicals and Reagents

Ethanol (AR grade 99% purity) was purchased from Sigma-Aldrich, Bangalore, India Millipore water (Milli-Q) used in the study was collected from Department of Nano Science and Technology. Crocin ($C_{44}H_{64}O_{24}$) was purchased from Sigma-Aldrich; Bangalore, India was used as the internal standard.

2.3. Extraction

The flower powder was subjected to conventional method of extraction. Flower powder of 20 g was macerated with 200 ml of ethanol; similarly 10 g powder was macerated with 130 ml of water. Both mixtures were kept in magnetic shaker for 48 hours. Later it was filtered, subjected to rotary evaporator (Heidolph Model-G3, Germany) for evaporation. The crude extracts thus obtained were weighed and stored for further analysis.

2.4. Quantification of Crocin Content

Crocin Estimation

Absorbance measurements were made in an Epoch microplate spectrophotometer (BioTek Intruments, Inc., Winooski, VT, USA) using a flat bottom 96-well quartz microplate (Hellma, Germany). Crocins concentration in each phase was determined by measuring the absorbance at 440 nm (Montalvo-Hernández B *et al*., 2012) [12].

A blank system (where water was used as sample) was prepared for each treatment and used as analytical blank for the corresponding phase (top or bottom). A calibration curve for crocins quantification was constructed in water (5-30 mg ml−1) with a correlation coefficient of 0.9909. The regression equations were Y=0.0922x+0.127 (R^2 =0.9909) where Y is the peak area ratio of analyte, and X is the concentration of the analyte (ug/g).

Table 1: Analysis of crocin content from the flowers of *Nyctanthes arbortristis*

Method of extraction	Type of solvent	Parmeters	Crocin content (mg/g) or in (g)
Conventional	Ethanol	20 g, 200 ml, 48 hours	3.81±0.09 g
	Water	10 g, 130 ml, 48 hours	3.18±0.03 g

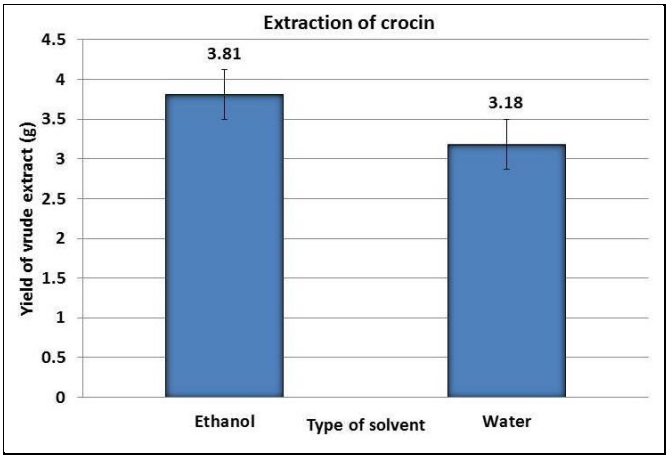

Fig 1: Quantification of crocin in flowers extracted from two different solvents - Ethanol and Water

2.6. Statistical Analysis

On the basis of single factor experiment, the major factors were carried out in triplicates. The Critical value were assessed separately for flowers of *N. arbortristis* Linn. Analysis of variance (ANOVA) was performed with Completely Randomized Design (CRD) The correlation co-efficient (R) were calculated to determine their relationship and *p* values < 0.05 are significant.

3. Results and Discussions

The recovery of bioactive glycosides-crocins from *N. arbortristis* is affected by more than one factor such as extraction technique and solvent, temperature and length of the process, and location of the compounds in the cell (Kyriakoudi A *et al.*, 2012) [9]. In conventional extraction, the yield from ethanol was found to be higher compared to water as a solvent (Fig 1A, 1B). However, we should consider the fact of lower efficiency, high energy consumption, long extraction time, thermal and hydrolytic degradation of some compounds are the major disadvantages of the conventional extraction techniques (Garavand *et al.*, 2019) [10]. For instance, monoterpenes are highly prone to chemical changes and experience considerable losses during solvent removal (Bayramoglu *et al.*, 2008) [11]. Therefore, this could be the reason for the slight reduction in the yield of ethanol extract when compared to water extract.

On the other hand, water is also found to be the best choice of modifier for crocetin sugar esters under MAE. This could be attributed to the fact that crocins are amphipathic molecules the sugar constituents gives them a polar character (Montalvo *et al.*, 2012) [12]. Since crocin possesses a sugar moiety, its solubility increases with increasing the solvent. Besides, by increasing ethanol concentration, the dissociation temperature of crocin arose. This can be ascribed to the amount of water present in the solvent. Since the specific heat of water (4.18 J/g °C) is more than that of ethanol (2.46 J/g °C), the increase in ethanol concentration reduced the solvent enthalpy at a constant temperature (Yang B *et al.*, 2009) [13].

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