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Research Article

Identification of Safranal in Volatile Oil Extracted from Tubular Calyx of *Nyctanthes arbor-tristis* : A Substitute to Saffron Aroma

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Abstract

Orange coloured tubular calyx of *Nyctanthes arbor-tristis* contains carotenoid resembling crocin in saffron. Etheral extract of the tubular calyx was investigated for the presence of safranal, which is main constituent in saffron responsible for bitter taste and aroma. HPTLC analysis of ethereal extract of the calyx and saffron indicated resemblance in Rf value and UV Spectrum. Presence of safranal in the volatile oil of the tubular calyx of *N. arbor-tristis* was confirmed by GCMS analysis. It can be concluded from the studies that the tubular calyx of *N. arbor-tristis* is not only the substitute for saffron colouring matter but also for aroma.

Keywords: *Nyctanthes arbortristis,* safranal, saffron aroma.

Introduction

Nyctanthes arbor-tristis is a holy plant which is planted in the gardens in India. The leaves of the plant are used for their antipyretic activity, while the stem bark is used for its antihelmentic activity [1]. The flowers are white in colour with a bright orange coloured tubular calyx. Methanolic extract of the flowers were also found to have analgesic, anti-inflammatory and antioxidant activities in preliminary studies [2-4]. Phytochemical investigations on the orange-red coloured dye obtained from the cold extraction of the tubular calyx indicated resemblance of thin layer chromatography (TLC) profile of the dye and the dye from 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, the pigment responsible for the red-orange colour of saffron [5]. Spectroscopic analysis confirmed that it is a carotenoid glycoside resembling crocin and the spectroscopic analysis confirmed the structure of aglycone to be crocetin [6].

Saffron is conferred as World's most expensive spice, hence is called as a luxury spice. It is usually adulterated with safflower stigmas coloured with synthetic dye to retain its colour. Crocin in saffron during storage, due to enzymatic action, yield D-glucose and Safranal molecule (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) which is a main component of the essential oil and responsible for the characteristic aroma of saffron [7-9]. Safranal forms about 70% of the total volatiles. An apocarotenoid called as Crocin is the colouring principle present both in saffron and in the calyx of *N. arbor-tristis*. Hence, there is a high probability of presence of these principles in the volatile oil of the flower of *N. arbor-tristis,* specifically orange coloured tubular calyx.

The flowers of *N. arbor-tristis* blossom at night and wither the next morning. The flowers perish within 20 minutes and are literally wasted due to their short life. Therefore, it was thought worthwhile to investigate if the orange coloured tubular calyx of *N. arbor-tristis* have 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. With this background, the present study was undertaken to investigate the presence of safranal in *N. arbor-tristis*.

Materials and Methods

Materials

The fresh flowers of *N. arbor-tristis* were collected from the gardens of the local area of Dombivli East, Dist. Thane, Maharashtra, India. The flowers were then washed under running tap water and the orange coloured tubular calyxes were separated manually from the petals. The flowers were authenticated at Khalsa college,Matunga,the voucher specimen (AN 0707142) is deposited in the institute . Pure saffron stigmas were purchased from Yucca Enterprises, Mumbai. The AR grades solvents viz. methanol, diethyl Ether, hexane, ethyl acetate and glacial acetic acid were purchased from Chemsol Enterprises, Mumbai.

Extraction of essential oil from *Nyctanthes arbor-tristis*

The calyxes were extracted with diethyl ether by macerating in dark for 6-8 hours at room temperature. The procedure repeated thrice to extract maximum content of volatile oil. The etheral extract was then filtered and was concentrated using Rotary Flash Evaporator.

Extraction of essential oil from stigmas of *Crocus sativus*

The essential oil was extracted from stigmas of *Crocus sativus* by macerating with Diethyl ether in dark till all the coloring matter is exhausted. The procedure repeated thrice to extract maximum content of volatile oil. The etheral extract was then filtered and was concentrated using Rotary Flash Evaporator.

Analysis of the Essential oil

HPTLC analysis of the ethereal extracts

Ether extracts of the calyx and saffron stigma were diluted and applied onto precoated silica gel GF - 254 plates (HPTLC plates, Merck KGaA) using a CAMAG Linomat V, Hamilton Syringe and the plate was developed in a hexane: ethyl acetate (9:1) solvent system. The plate was then scanned in the wavelength range of 200-600 nm using a CAMAG Scanner 3 (Version 1.14.28). The analysis of volatile oil was carried out at 310 nm.

GC-MS analysis of the ethereal extracts of the tubular calyx and saffron

The etheral extracts of tubular calyx of *N. arbor-tristis* and saffron stigma were analyzed using a GC-MS instrument (GCD - HP1800A). The fresh etheral fractions were submitted to IIT, Mumbai to record GC-MS spectra. The GC-MS system was equipped with capillary column of HP-5 (30 m x 0.25 mm; film thickness 0.25 µm) and a splitless injector. The oven temperature was programmed from 100ºC to 200ºC at the rate of 10ºC/minute and held at this temperature for 3 minutes and then further increased to 280ºC at 15ºC/minute and held

at this temperature for 3 minutes. The injector and detector temperature were set at 200ºC and 280ºC, respectively. A volume of 0.1 µl of the ether fraction was injected into GC-MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The quadrapole mass analyzer contained an electron impact ion source with filament potential of 70 eV. The chemical components of the ether fraction were identified by comparing their mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology).

Result and Discussion

HPTLC analysis of the etheral extracts of orange coloured tubular calyx of *N. arbortristis* and saffron showed that the Rf value of safranal in saffron matches with the Rf value of etheral extract of *N. arbortristis* (Table 1 and Figure 1).

Table 1. Comparison of HPTLC chromatogram of the etheral extracts of tubular calyx of *N. arbor-tristis* and Saffron stigma

Name of compound	Area	Rf value
Saffron extract	791.4	0.46
N. arbor-tristis	968	0.46

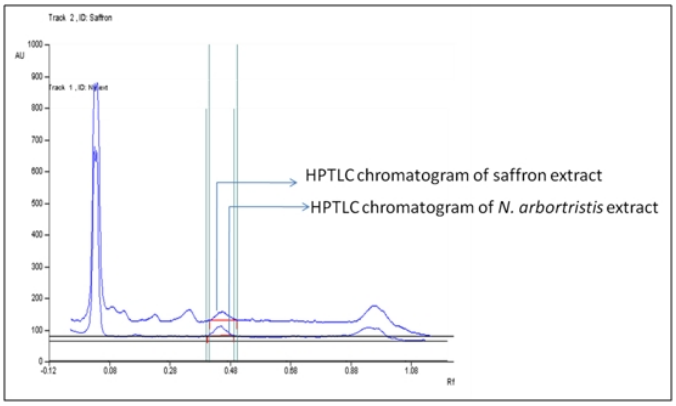

Figure 1. Overlay of HPTLC chromatograms of the etheral extracts of tubular calyx of N. arbor-tristis and Saffron stigma

The wavelength scan of spot corresponding to Rf 0.5 was referred as safranal, was found to be coinciding in both the tracks of saffron extract and the *N. arbor-tristis* extract [10] (Figure 2). Both the extracts were further subjected to GC-MS analysis for confirmation of safranal. GC-MS analysis of volatile oil of flower calyx of *N. arbortristis* indicated the presence of 10 compounds (Figure 3). A comparative data from gas chromatograms of the etheral extracts of saffron stigma and tubular calyx of *N. arbor-tristis* is given in Table 2.

Table 2. Comparison of data from gas chromatograms of the etheral extracts of saffron stigma and tubular calyx of *N.arbor-tristis*

Name of compound	Retention time (min)	Name of constituent present	Area %
Saffron extract	19.4	safranal	23.54
N. arbortristis extract	20	safranal	1.55

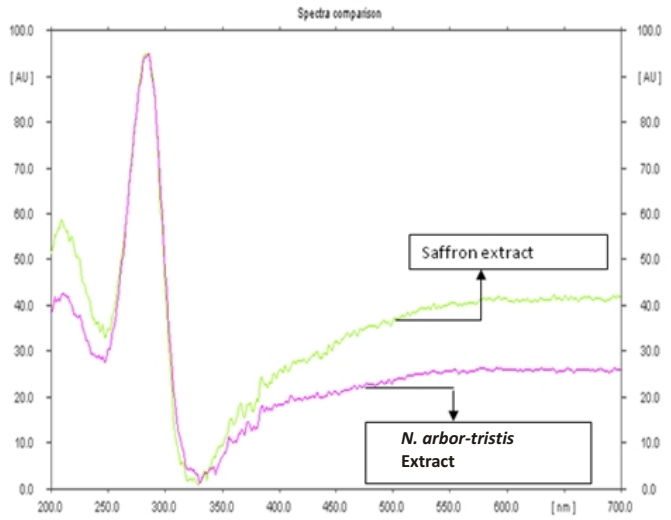

Figure 2. Overlay of wavelength scans of the spot corresponding to Rf 0.46 of ether extracts of saffron and tubular calyx of *N. arbor-tristis*

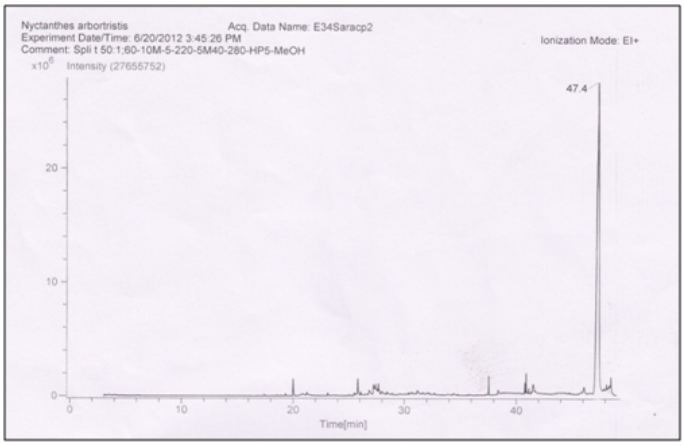

Figure 3. Gas chromatogram of the ether extract of tubular calyx of *N.arbor-tristis*

The MS spectra of only one peak with retention time (20.0 min) of *N. arbor-tristis* was observed to be matching with MS spectra of saffron peak with retention time (19.4 min) (Figure 4-6).

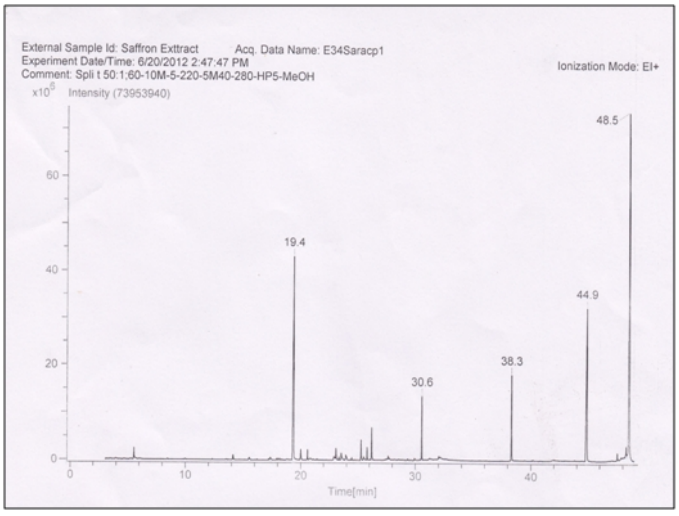

Figure 4. Gas chromatogram of the etheral extract of saffron stigma

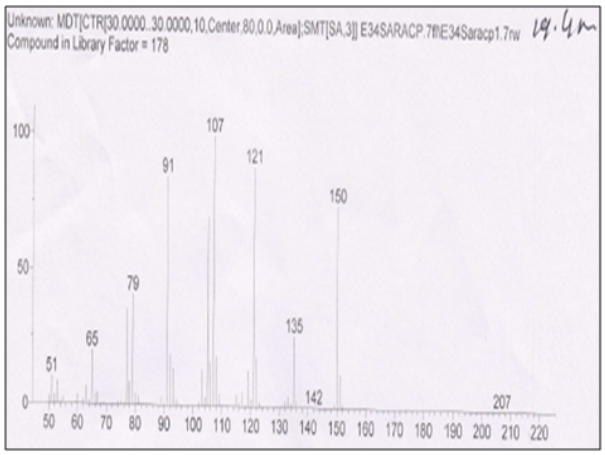

Figure 5. Mass spectrum of the component with retention time 19.4 of the etheral extract of saffron stigma

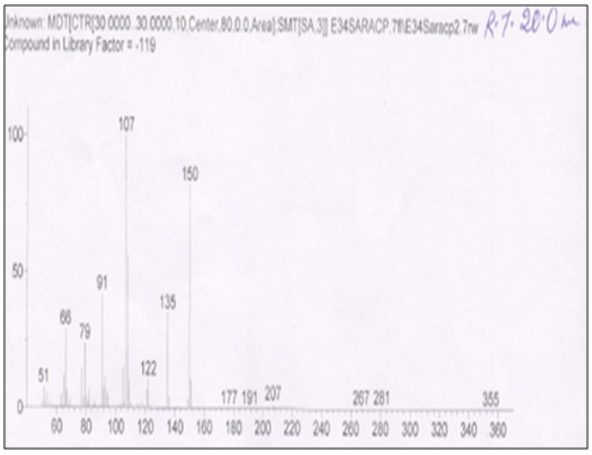

Figure 6. Mass spectrum of the component with retention time 20 minutes of the etheral extract of tubular calyx of *N. arbortristis*

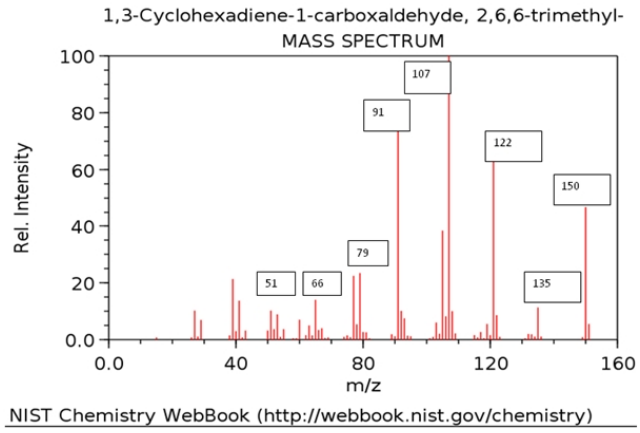

Figure 7. Mass spectrum for safranal from NIST library

The data obtained from the GC-MS analysis was then compared with the database from NIST Library [11]. The probabilities given for the component with retention time 19.4 (Saffron extract) and 20.0 minutes (*N. arbor-tristis* extract) indicated that it could be Safranal, with molecular formula-$C_{10}H_{14}O$, with molar mass 150.21 g/mol and λmax value at 310 nm. Therefore, it was concluded that the volatile oil of tubular calyx of *N. arbortristis* may be containing the compound like Safranal (Figure 7).

Conclusion

From the results obtained it was concluded that the orange colour tubular calyx of *N. arbor-tristis* contains safranal having molecular formula $C_{10}H_{14}O$. Thus *N. arbor-tristis* being easily available and cost effective can be used as a substitute to saffron not only for colour but also for its aroma.

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Declaration

The authors report no conflicts of interest

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