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Scrutinizing variation in amount of Taraxerol amongst the floral morphotypes of *Clitoria ternatea* L. and determination of the best producer morphotype.

KEYWORDS

Clitoria ternatea, Morphotypes, Taraxerol, Terpenoids, HPTLC, FT-IR.

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ABSTRACT *Clitoria ternatea L., a perennial twiner under the family Fabaceae and provided with different colours and forms of flower, is also the source of a host of valuable chemicals of immense medicinal virtues. Taraxerol is such an anti-depressant agent with anxiolytic, nootropic, COX inhibitor and other properties of clinical significance occurring in this plant; in greater amount in roots. The present work with an objective of scrutinizing any relationship between the varied forms of flower and the amount of taraxerol could identify plants bearing blue flower to produce more taraxerol than white coloured flower, double type superior over single type irrespective of colour of petal and the polyadelphous arrangement of stamen to be associated with greater amount of taraxerol than other types. In consideration of all these traits, in combination, plants bearing blue double type of flower with polyadelphous stamen were found to yield the greatest amount of it.*

Summery:

Clitoria ternatea L. is known to have taraxerol as a valuable biochemical of immense medicinal significance amongst a host of such chemicals available in it. The species having well known variation in flower colour and arrangement of petals also shows diversity in arrangement of stamens. Thus, on the basis of two contrast colours of blue and white as well as single and double types based on petal arrangement in conjunction with stamen arrangement as diadelphous, polyadelphous and solitary five floral forms of flower of the species have been considered here. Specific nature of relationship between these morphotypes and taraxerol content has been determined here. Plants with blue double flower and polyadelphous stamens have been noted to possess greatest amount of taraxerol amongst all five types. Blue flower plants have greater amount of the active principle than the white flowered plant, double type has greater amount than the single type and polyadelphous type has greatest amount in comparison to other types of stamen arrangement.

Introduction:

The species *Clitoria ternatea* L. is a perennial climber, up to 2-3 m in height, growing wild and also in gardens, bearing blue or white flowers resembling a conch-shell. The plant grows as a foliage rich thick bush yielding profuse flowers throughout the year. The species is popular in India and Bangladesh for the religious use of flower and also due to some medicinal uses. Ethno-medicinal uses of this plant have been recorded for different ethnicities and races of humankind throughout the world. The major phytochemical constituents found in *C. ternatea* L. are two pentacyclic triterpenoids viz. Taraxerol and Taraxerone (Banerjee & Chakravarti, 1963, 1964). Lots of research are going on throughout the world to identify its pharmacological applications. Taraxerol has been proved to be a potent drug which can enhance memory and learning capacity. It has anti-depression, anxiolytic, nootropic and anti-stress activity. Taraxerol can be used effectively to treat Alzhiemer's and Parkinsonism (Ngo & Li, 2013) due to its acetylcholinesterase inhibition property (V. Kumar, Mukherjee, Pal, Houghton, & Mukherjee, 2007). Being a triterpenes it is also known to enhance the inhibitory effects of anti-cancer drugs (Yamai et al., 2009). Taraxerol is known to induce apoptosis, acts as COX inhibitor (Ur Rehman et al., 2013) and possesses anti-microbial potential (Singh, Sahu, & Sharma, 2002). Taraxasterol is anti-allergic (Zhang et al., 2013), anti-oxidant (Gallova, Horvathova, & Grancai, 2007), and anti-inflammatory (Zhang, Xiong, & Liu, 2012). Taraxerol is also known to benefit in diabetes (Sangeetha, Shilpa, Jyothi Kumari, & Lakshmi, 2013). Both the compounds are also known to exhibit antagonistic property against snake venom (Mors, Nascimento, Pereira, & Pereira, 2000).

As in consideration of WHO (World Federation for Mental Health & WHO, 2012) anxiety, stress, depression and other neurological disorders like Alzheimer's and Parkinson disease are posing serious threat in near future, the organization is promoting research for screening out natural drugs with anti-depressant activity, specially the drugs with serotonin uptake inhibitor or acetylcholinesterase inhibitor property. In this regard a search of the herbal sources of Taraxerol, as well as the source with higher amount of it would be quite prospective in consideration of its increasing demand in the world market.

Flowers of this species are mainly of two types on the basis of colour, - blue and white, besides some rarely met colours like violet, pink etc. Often a critical observation helps to demarcate finer variations in the hue of blue colour of petals and on veins of petals. A further variation in the shapes of petals and arrangement thereof make distinctive patterns, often colloquially designated as single and double, though the number of petal remains same. Flowers also vary by the shape of petals and thus both blue and white varieties have single and double type of flowers. Fantz. (1977, 1990) classified the species *C. ternatea* L. into varieties and forms mainly on the basis of flower morphology, colour of the flower, and arrangements of stamens, as below:

Flowers papilionaceous, solitary, stamens diadelphous

Var. Ternatea

Flowers azure to dark blue, standard yellow to white medial strip and basally purplish veins. Peduncle 1/axil, 0.5 cm long.

f. fasciculata Fantz.

Flowers white, solitary, rarely 2-3 flowered with greenish to greenish white medial strip on standard and basally purplish veins. Peduncle 1/axial, 0.5 to 1.5 cm long.

f. albiflora. (Voigt) Fantz.

Flowers "double", actinomorphic, petals 5 standard like, stamens ten free or united in bundles.

Var. Pleniflora

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Flowers blue. Peduncle solitary rarely paired stamens free.

f. pleniflora Fantz.

Flowers white, solitary, rarely 2 flowered, stamens free.

f. leucopetala Fantz.

Flowers blue, solitary, stamens subpolyadelphous

f. subpolyadelpha Fantz.

Root tissue of *C ternatea* L. contains considerable amount of taraxerol (Venkatesan Kumar, Mukherjee, Kumar, Mal, & Mukherjee, 2008). Though morphological polymorphism of *C. ternatea* L. is quite apparent, any work delving into the relation between floral morphotypes and taraxerol productivity is absent. Till now no study has been conducted to find the variations of taraxerol production by different floral morphotypes of *C. ternatea* L. However, any information on a relation between these morphotypes and difference in amount of taraxerol productivity, if existing, would be of much commercial worth.

Materials and Methods

Materials:

Five different floral morphotypes of *C. ternatea* L. were collected from Midnapore, West Bengal, India. Stock plants were identified accordingly using the identification key by Paul R. Fantz. (1977). Five plants of each type were taken as replica to represent each of the types. Moreover, vegetative cloning of all stock plants was done for the purpose of increasing the amount of plant material required to execute the quantitation and related matters. Five floral morphotypes of *C. ternatea* L., considered under present experiment are [1. Var. Ternatea *f. fasciculata* Fantz. (**BS**); 2. Var. Ternatea *f. albiflora*. (Voigt) Fantz. (**WS**); 3. Var. Pleniflora *f. leucopetala* Fantz. (**WD**); 4. Var. Pleniflora *f. pleniflora* Fantz. (**BD2**); 5. Var. Pleniflora *f. subpolyadelpha* Fantz. (**BD1**)] Estimation of taraxerol was conducted for all these five types. Fresh roots of 45-50 days old plants were used in the experiments. Five replications of each samples were used for the quantitation of taraxerol.

3b-Taraxerol (Analytical standard) was used as the standard for analyses which was obtained from Sigma-Aldrich, CAS Number 127-22-0. Other analytical grade solvents used in experiments were of E-Merck make.

Preparation of sample:

Roots from the plants were collected freshly and dried under shade. After complete drying roots were powdered using mixer grinder. Ten grams of powdered root from each sample was taken for extraction. Powdered root was extracted with 50ml. of 70% ethanol. Powdered root was extracted with 50ml. of 70% ethanol. Root powder was soaked for 48 hours at room temperature then the hydroalcoholic extract was strained out. This process was repeated and monitored for the presence of taraxerol by TLC analyses and continued till the presence of taraxerol within the hydroalcoholic extract (Venkatesan Kumar et al., 2008). After an exhaustive extraction all hydroalcoholic extracts were combined and used for further processing. The combined extract was filtered through Whatman qualitative filter paper no. 1 and evaporated to dryness under reduced pressure at 45°C temperature in a rotary evaporator. Remnant was collected and finally dried under high vacuum in Speed Vac. Concentrator and afterwards in lyophilizer to furnish the final extract. Then the dark brownish residue was dissolved in methanol and filtered through Whatman qualitative filter paper no. 1, pore size 11 m (Maidstone, UK). The volume was adjusted with methanol to get the final concentration of 1mg. $ml.^{-1}$ This final preparation was used for HPTLC analyses.

Chromatographic conditions and Instrumentation:

HPTLC was performed using Camag (Muttenz, Switzerland) HPTLC system which includes a Camag twin-trough plate development chamber, a Linomat IV sample applicator, Camag TLC Scanner 3 and integrated winCATS software. Silica Gel 60 F_{254} (E. Merck) Aluminium backed HPTLC plates, prewashed with methanol were used to carry out the analyses. A working standard (100µg $ml.^{-1}$) of 3β-Taraxerol was prepared using methanol, to use as working standard. Six different concentrations of this solution were applied using a Linomat IV sample applicator. Samples were applied on the plates at about 1 cm above the edge using a bandwidth of 10 mm and distance between tracks of 5 mm (Venkatesan Kumar et al., 2008). The chromatogram was developed up to 80 mm under chamber saturation conditions with *n*-pentane and ethyl acetate (80:20 v/v). A solution of *n*-pentane and ethyl acetate (80:20 v/v) was used as mobile phase in Camag twin-trough chamber. After completion of HPTLC run plates were air dried and scanned with the help of Camag TLC Scanner 3 using a detector wavelength of 366nm. Spot of taraxerol were then scraped and recrystallized to use for further FT-IR and EIMS analysis of the compound.

Six different concentrations of taraxerol were applied to obtain the calibration plot. Using selected mobile phase the R_f of taraxerol was found to be 0.47. A scatter plot was obtained by plotting scanned data of absorbance area (AU) against Y axis and the amount of taraxerol (ng) against X axis. Thus, the calibration plot was made. R^2 value 0.9986 is indicating strong linear dependence of absorbance area on concentration. The linear equation of calibration curve is y = **9.159**x + **192.39,** where y representing the value of absorbance area and x is the value of concentration. The taraxerol concentrations unknown samples of *C. ternatea* L. root extracts were calculated using this equation. 10 l of each aliquot of the extract (1 mg $ml.^{-1}$) was applied along with 10 l of the standard solution (100 g $ml.^{-1}$). After chromatography, the amount of taraxerol present in the extract was calculated using the equation obtained from the calibration plot.

Isolation of taraxerol:

Shade dried plant roots were extracted with ethanol till the presence of taraxerol at 60°C temperature and filtered using Whatman qualitative filter paper. The filtrate was evaporated under reduced pressure in a rotary evaporator at 45°C. The concentrated filtrate was further evaporated and dried using lyophilizer yielding a dark brownish residue. The resultant product was then suspended in water and subsequently extracted using hexane, chloroform, ethyl acetate and *n*-butanol. The combined fraction of chloroform and hexane was then subjected to chromatography on a silica gel column, using methanol-chloroform (from 0:100 to 100:0, v/v) as an eluting agent. Five fractions (A-E) were obtained. The fraction A was chromatographed on silica gel column using hexane-ethyl acetate (80:20, v/v) as an eluent to yield the desired compound. The isolated compound was crystalized and used for FT-IR and EI-MS.

FT-IR analysis:

Fourier transform infrared spectroscopy (Thermo Scientific Nicolet 1S5 FTIR) was used to analyse the recrystallized compounds obtained from scrapped spots as well as the compound obtained from isolation process. Spectrum was recorded in the ranges between 600nm-4000nm by KBr pellet technique.

EI-MS analysis:

Electron Ionization Mass Spectrometry (EI-MS) of the isolated compound as well as of the scrapped compounds were performed following standard protocol, for further characterization of the compounds.

Statistical analysis:

Statistical analyses were performed with the help of IBM SPSS STATISTICS version 21 and Microsoft Excel.

Results and Discussion:

Volume - 7 | Issue - 1 | January - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

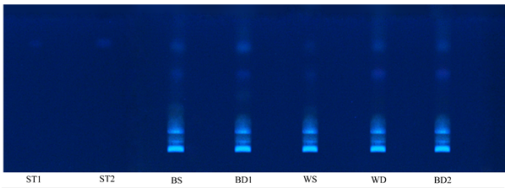
Powdered roots of each plants were analysed in HPTLC for separation and quantification of taraxerol. Each and every sample produced a very discrete spot against standard on HPTLC plate at R_f 0.47. The mobile phase resolved the taraxerol very efficiently from other compounds. The highest amount of taraxerol was obtained from BD1: Var. Pleniflora *f. subpolyadelpha* Fantz. (Blue double with polyadelphous stamens). Roots of BD1 contained more than 12mg g^{-1} of taraxerol, which is nearly double to that of BD2: Var. Pleniflora *f. pleniflora* Fantz. (Blue double with

Table 1. Taraxerol content of different floral morphotypes of C. ternatea L.

Sample no.	Morphotypes	Absorbance area average (AU)	Taraxerol content average (mg g^{-1})
1	BD1	1320.24 ± 35.35	12.313310 ± 0.39
2	BD2	772.2 ± 40.79	6.3300802 ± 0.45
3	WD	769 ± 22.52	6.2951438 ± 0.25
4	WS	485.52 ± 23.34	3.200249 ± 0.25
5	BS	634.48 ± 27.61	4.8265208 ± 0.30

solitary stamens) and WD: Var. Pleniflora *f. leucopetala* Fantz. (White double with solitary stamens). White single (WS) i.e. Var. Ternatea *f. albiflora*. (Voigt) Fantz. was found to contain the lowest amount of taraxerol (3.20 mg g^{-1}). Taraxerol content of blue single (BS) i.e. Var. Ternatea *f. fasciculata* Fantz. was slightly higher to that of WS.

The purified and crystalized compound was obtained as a colourless amorphous powder. Melting point of the compound was determined to be 282°C. FT-IR spectra of the identified spot showed absorption band at 3445 cm^{-1} and 3210 cm^{-1}, were due to stretching vibration of OH (Inter molecule bound). Peak at 3055 cm^{-1} was derived from the stretching vibration of –



1. BS: Var. Ternatea *f. fasciculata* Fantz. (Blue single); **2. WS:** Var. Ternatea *f. albiflora.* (Voigt) Fantz. (White single); **3. WD:** Var. Pleniflora *f. leucopetala* Fantz. (White double with solitary stamens); **4. BD2:** Var. Pleniflora *f. pleniflora* Fantz. (Blue double with solitary stamens); **5. BD1:** Var. Pleniflora *f. subpolyadelpha* Fantz. (Blue double with polyadelphous stamens).

Figure1. HPTLC plate scanned at 366 nm.

CH of alkene. Absorption ribbons at 2975 cm^{-1}, 2950 cm^{-1} and 2875 cm^{-1} were formed due to the stretching vibrations of –CH from alkanes. A small hump at the area of 1802 cm^{-1} was formed due to overtone of CH bending vibration. Peaks at 1668 cm^{-1} and 1650 cm^{-1} derived from C=C (Trisubstituted Alkene) stretching. At 1440 cm^{-1} of IR spectrum an absorption ribbon was formed due to CH bending of Methyl group of alkene. CH bending of Gem dimethyl (Alkene) were represented by peaks at 1385 cm^{-1} and 1365 cm^{-1}. Bending vibration of OH group produced absorption ribbon at 1330cm^{-1} and 1310cm^{-1}. An absorption peak at 880cm^{-1} was appeared due to aromatic –CH bending. C=C bending of trisubstituted alkene produced

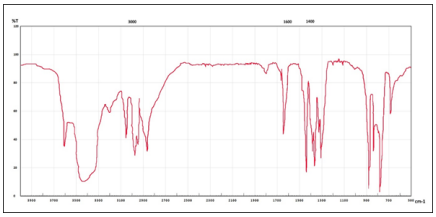


Figure 2. FT-IR spectrum with characteristics peaks of taraxerol.

peak at 840 cm^{-1} and the absorption ribbons at 790 cm^{-1} and 690 cm^{-1} were derived from bending vibration of aromatic CH.

EI-MS spectrum of isolated compound gave diagnostic peaks at *m/z* 302.27, 287. 24, 269.22, 204.18, 189.17. It produced the molecular peak at 426.601(M-$C_{30}H_{50}O$) *m/z* [M^+] (calcd. 426.7174). FT-IR and EI-MS spectrums confirmed the identity of isolated compounds as Taraxerol ($C_{30}H_{50}O$).

It is apparent from the findings that arrangement of petals and the nature of cohesion of stamens may be used as index characters to identify higher productive form of C. ternatea L. in terms of taraxerol production. Primarily plants with double type flower was identified as better productive form than that of single type. Moreover, the plants with polyadelphous stamens were determined to be the highest productive even amongst the double flowered varieties.

Conclusion:

The study reveals that the floral morphotypes of *Clitoria ternatea* L., distinguished by the dint of not only the colour and arrangement of petals but also the arrangement of stamens, show variation in the amount of taraxerol content in a regular manner in accordance with the types. Taraxerol content was found to be the highest in *C. ternatea* L. Var. Pleniflora *f. subpolyadelpha* Fantz. (Blue double with polyadelphous stamens) among all floral morphotypes of *C. ternatea* L. studied here.

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References:

1. Banerjee, S. K., & Chakravarti, R. N. (1963). Taraxerol from Clitoria ternatea. Bull Calcutta School Trop Med, 11, 106–107.
2. Banerjee, S. K., & Chakravarti, R. N. (1964). Taraxerone from Clitoria ternatea. Bull Calcutta School Trop Med, 12, 23.
3. Fantz, P. R. (1977). A MONOGRAPH OF THE GENUS Clitoria (LEGUMINOSAE: GLYCINEAE). University of Florida. Retrieved from http://ufdc.ufl.edu/AA00003919/00001
4. Fantz, P. R. (1990). Clitoria (Leguminosae). Antillarum. Moscosoa ., 152–166.
5. Gallova, J., Horvathova, M., & Grancai, D. (2007). Taraxasterol inhibits the peroxidation of egg yolk phosphatidylcholine in liposomes. Acta Facul Pharm Univ Comeniane, 3, 70–77.
6. Kumar, V., Mukherjee, K., Kumar, S., Mal, M., & Mukherjee, P. K. (2008). Validation of HPTLC method for the analysis of taraxerol in Clitoria ternatea. Phytochemical Analysis, 19(3), 244–250. https://doi.org/10.1002/pca.1042
7. Kumar, V., Mukherjee, K., Pal, B. C., Houghton, P. J., & Mukherjee, P. K. (2007). Acetylcholinesterase inhibitor from Clitorea ternatea. Planta Medica. 73:973–974., 73, 973–974.
8. Mors, W. B., Nascimento, M. C., Pereira, B. M., & Pereira, N. A. (2000). Plant natural products active against snake bite--the molecular approach. Phytochemistry, 55(6), 627–642. https://doi.org/S0031942200002296 [pii]
9. Ngo, S. T., & Li, M. S. (2013). Top-leads from natural products for treatment of Alzheimer's disease: docking and molecular dynamics study. Molecular Simulation, 39(4), 279–291. https://doi.org/10.1080/08927022.2012.718769
10. Sangeetha, K. N., Shilpa, K., Jyothi Kumari, P., & Lakshmi, B. S. (2013). Reversal of dexamethasone induced insulin resistance in 3T3L1 adipocytes by 3 -taraxerol of Mangifera indica. Phytomedicine, 20(3–4), 213–220. https://doi.org/10.1016/j.phymed.2012.10.011
11. Singh, B., Sahu, P. M., & Sharma, M. K. (2002). Anti-inflammatory and antimicrobial activities of triterpenoids from Stribolanthes Callosus nees. Phytomedicine, 9, 355–359.
12. Ur Rehman, U., Shah, J., Ahmed Khan, M., Raza Shah, M., Ishtiaq, & Khan, I. (2013). Molecular docking of taraxerol acetate as a new COX inhibitor. Bangladesh Journal of Pharmacology, 8(2), 194–197. https://doi.org/10.3329/bjp.v8i2.14167
13. World Federation for Mental Health, & WHO. (2012). DEPRESSION: A Global Crisis World Mental Health Day, October 10, 2012. Retrieved from www.who.int/mental_health/management/depression/wfmh_paper_depression_wmhd_2012.pdf
14. Yamai, H., Sawada, N., Yoshida, T., Seike, J., Takizawa, H., Kenzaki, K., … Tangoku, A. (2009). Triterpenes augment the inhibitory effects of anticancer drugs on growth of human esophageal carcinoma cells in vitro and suppress experimental metastasis in vivo. International Journal of Cancer, 125(4), 952–960. https://doi.org/10.1002/ijc.24433
15. Zhang, X., Liu, J., Xiong, H., Cheng, Y., Cui, C., Zhang, X., & Xu, L. (2013). Effects of taraxasterol on ovalbumin-induced allergic asthma in mice. Journal of Ethnopharmacology, 148(3), 787–793. https://doi.org/10.1016/j.jep.2013.05.006
16. Zhang, X., Xiong, H., & Liu, L. (2012). Effects of taraxasterol on inflammatory responses in lipopolysaccharide-induced RAW 264.7 macrophages. Journal of Ethnopharmacology, 141(1), 206–211. https://doi.org/10.1016/j.jep.2012.02.020