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A quinoline alkaloid rich *Quisqualis indica* floral extract enhances the bioactivity

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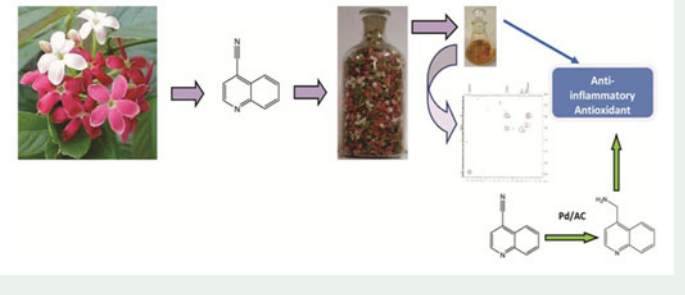
ABSTRACT

A volatile alkaloid quinoline-4-carbonitrile (QCN) was isolated from the floral extract of *Quisqualis indica*. Major compounds were *trans*-linalool oxide (1.0, 4.5%), methyl benzoate (1.0, 4.0%), 2,2,6-trimethyl-6-vinyl-tetrahydropyran-3-one (7.4, 17.8%), 2,2,6-trimethyl-6-vinyl-tetrahydropyran-3-ol (1.0, 1.2%), (*E,E*)-α-farnesene (29.1, 16.1%), QCN (5.7, 1.3%) in live and picked flowers, respectively. Flower compositions were altered due to change in enzymatic reaction at the time of picking. Some rearrangements of oxygenated terpenoids occurred in the process of hydrodistillation to obtain essential oil. Chemical synthesis of QCN and its selectively reduced products derived from QCN were prepared through green reaction process. The catalytic modification of QCN has produced quinoline-4-methylamine; the later compound has shown enhanced bio-activities. QCN and floral extract (absolute) have shown potential anti-inflammatory and antioxidant activities. Besides, floral absolute has shown significant anti-inflammatory and antioxidant activities due to improved QCN (19.7%) content to synergize amongst terpenoids and benzenoids as compared to the essential oil with 1.1% of QCN.



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1. Introduction

Quisqualis indica L syn. *Q.densiflora* (family *Combretaceae*) is a large woody scandent shrub and a hardy creeper to provide profuse shade around the house (Anon 1991). This plant is native to Indo-Malaysian region as well as in tropical Africa. In general, it is being cultivated throughout the India as well as in China (Yan et al. 2018). It is planted in gardens for the white-red colored flowers and particularly blooming generously in pleasant weather during the months of March–May (Spring) and September–November (Autumn). Flowers blossom in the evening as white colored flowers and gradually changing to red by afternoon. Flowers have a distinguishing mild refreshing fragrance soothing into the environment during evening hours to late night. The floral aqueous-alcoholic extract had shown 90.2% of antioxidant activity in 500 mg/L solution with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Afify and Hassan 2016). Recently, Yan et al. (2016) reported 35 compounds based on the computer matching of mass spectra. Rout et al. (2008) had reported the comparison of concrete, absolute and essential oil of *Q.indica* flowers. There are 34 compounds identified including quinoline-carbonitrile through GC/MS analysis. Presently, fresh flowers are collected from CSIR-CIMAP campus (Accession No: 83008) for preparation of pentane extract (concrete) (Figure S1), and essential oil (Figure S2). Quinoline-carbonitrile is extracted upto 15% in concrete as compared to 1.1% in essential oil. However, its identification was only tentative and the exact isomer could not be ascertained. There was only report available on isolation of quinoline-4-carbonitrile (QCN) from Fungus *Xylaria sp*. BCC 9653 (Pongcharoen et al. 2007). Hence, thorough characterization work of this compound was undertaken (Figure S3). Furthermore, the extracts, isolated quinoline-carbonitrile and its derivatives from *Q. indica* flowers were investigated for *in-vitro* anti-inflammatory activity. Flower volatiles were determined by headspace-solid phase microextraction (HS-SPME) analysis. Due to its excellent fragrance profile as well as bioactivities (anti-inflammatory and antioxidant) of floral extract or its pure molecule, this flower may be useful in various pharmaceutical preparations.

2. Results and discussion

2.1. HS-SPME analysis of live and picked flowers

Headspace volatiles compositions of live and picked flowers contained mono-terpenoids (9.2–9.6%, 22.3–26.2%) and sesquiterpenoids (28.3–30.6%, 15.1–17.0%), respectively (Figure S4-S5, Table S1). In particular, *trans*-linalool oxide (4.2-4.8%) and 2, 2,6-trimethyl-6-vinyl-tetrahydropyran-3-one (14.7-19.2%) were significantly enhanced in picked flowers. Linalool was not detected in live flowers, but it was found accumulated upto 1.2% in picked flowers. In a similar study, Mookherjee et al. (1990) had studied a number of flowers and reported that linalool was significantly increased (up to 10 fold) in picked flowers. Interestingly, linalool (8.0%) was found higher percentage in essential oil, whereas it remained very low (0.2%) in the absolute. It is predicted that the enzymatic reaction after flowers picking followed by hydrodistillation at higher temperature probably increases linalool percentage in essential oil (Lawrence 1995). In contrast, live flowers contained improved percentage of (*E,E*)-α-farnesene

(28.0–30.3%) as compared to the picked flowers (15.0–16.8%). The present findings are in good agreement with reported work by Mookherjee et al. (1990). Importantly, QCN was detected with higher percentage in the live flowers (5.6–5.8%). It was also observed that the live flowers contained higher percentage of sesquiterpenes (29.5%) and QCN (5.7%) with pleasant aroma; hence, it precisely attracts the pollinator for healthy pollination (Yan et al. 2016). Variations in compositions of live and picked flowers are attributed to the enzymatic reaction and also impulsive shock arises due to detachment of flowers from the plant (Xu et al. 2018). Thus, picked flowers possess a modified aroma profile (Mookherjee et al. 1990).

2.2. Comparison of HS-SPME with the extracts

Percentage yields of the absolute and essential oil were 0.37% and 0.015%, respectively. The representative chromatogram of HS-SPME is given in Figure 5. It has been observed that the HS-SPME composition was quite different from that of essential oil. Pyranoid form a linalool oxide (2,2,6-trimethyl-6-vinyl-tetrahydropyran-3-ol) was present upto 1.3% in headspace contributing to top-note fragrance profile emitted by the flowers. This pyranoid form is very sensitive to heat in the presence of water, which resulted in rearrangement to linalool oxide during distillation (Figure S6). Other compounds identified were 2,2,6-trimethyl-6-vinyl-tetrahydropyran-3-one (5.5%) and linalool (8.0%) in the essential oil. Similarly, low linalool (1.2%) percentage in the flower headspace was observed but 2,2,6-trimethyl-6-vinyl-tetrahydropyran-3-one was accumulated up to 19.5%. Besides, furanoid form of *trans*- and *cis*-linalool oxides were identified in the essential oil due to their comparatively stable structures (Table S2). It has been reported that aromatic compounds exist in glycosidically bound form and at a specific developmental stage of flower, the floral parts follow a controlled enzymatic hydrolysis mechanism to release the terpenoids/benzenoids (Lawrence 1995). Thus, essential oil was not replicated the aroma profile of live flowers and QCN was isolated only in 1.1% in the essential oil. It was due to its lesser volatility with water in the process of hydrodistillation; however, it was recovered to 15% in the extract (Rout et al. 2008). Besides waxy materials, the absolute composition might be compared with HS-SPME compositions of flowers. Absolute was improved form of the extract contained 19.7% of QCN along with 42.1% of non-volatile wax like materials. Interestingly, alkaloids with structure relationship with QCN were not detected in LC-QTOF LC/MS analysis (Figures S7 and S8). Furthermore, quinoline-carbonitrile isolation and characterization was carried out using extensive NMR experiments.

2.3. Spectroscopic analysis of QCN

The yield of quinoline-carbonitrile was 430 mg from 4 g absolute. Elemental analysis indicated the percentage of C (79.1%), H (3.9%) and N (16.6%), the empirical molecular formula is $C_{10}H_6N_2$. FT-IR spectrum illustrated absorption at 2231 cm^{-1}, which confirms the presence of characteristic CN group (Figure S9). HR-MS analysis revealed that the molecular mass of M + H was 155.0604 having molecular formula $([C_{10}H_6N_2]+H)^+$ (Figure S10). Therefore, molecular formula of the compound was $C_{10}H_6N_2$.

Characteristic electron impact (EI) masses are molecular ion peak (*m/z* 154) along with *m/z* 127 and *m/z* 100 arising by loss of two successive HCN units as illustrated in the structure of quinoline-carbonitrile or isoquinoline-carbonitrile (Figure S11). ^{1}H-NMR (500 MHz, $CDCl_3$) for six protons viz. doublet (d) at δ 9.05 (1H), a double doublets (dd) at δ 8.22 (2H), a doublet of doublets of doublets (ddd) at δ 7.88 (1H), another ddd at δ 7.79 (1H) and d at δ 7.77 (1η) (Figure S12). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 115.5, 118.7, 124.8, 124.9, 125.8, 129.2, 130.4, 131.2, 148.1, 149.4 (Figure S13). Above analysis indicated that the probable structure would be one of the six possible isomers of quinoline-2-carbonitrile, quinoline-3-carbonitrile, QCN, quinoline-5-carbonitrile, quinoline-8-carbonitrile and isoquinoline-1-carbonitrile (Figure S14).

^{1}H-^{1}H-COSY shows a ddd coupling of aromatic protons indicating the presence of two ddd adjacent protons (Figure S15). Therefore, it ruled out the probable like quinoline-5-carbonitrile and quinoline-8-carbonitrile. Presence of downfield signal at δ 9.05 splitting to a doublet due to adjacent aromatic proton cannot be expected in isoquinoline-1-carbonitrile and leaving only quinoline-2-carbonitrile, quinoline-3-carbonitrile and QCN for deciding the structure. Further, synthesized quinoline-2-carbonitrile was used for spectral comparison. Its ^{1}H-NMR showed signals at δ 8.32 (d, 1H, $J = 8.4H_Z$, Ar-H), δ 8.18 (d, 1H, $J = 8.6H_Z$, Ar-H) , δ 7.90 (d, 1H, $J = 8.4H_Z$, Ar-H), δ 7.85 (ddd, 1H, $J = 8.6, 6.9, 1.5H_Z$, Ar-H), δ 7.71 (d, 1H, $J = 8.4H_Z$ Ar-H), δ 7.70 (ddd, 1H, $J = 8.2, 6.8, 1.0H_Z$ Ar-H) (Figure S16). ^{1}H-NMR of quinoline-2-carbonitrile and the expected structure are quite different. Hence, the structure may be either quinoline-3-carbonitrile or QCN. In case of quinoline-3-carbonitrile, proton attached at C-2 will experience electron with-drawing effect not only from the C = N of ring nitrogen but also from the CN group at 3-position. Consequently, this proton is expected at downfield farther to δ 9.05 as rather a singlet. Even if it couples with proton at C-4, *J* values will be about 2 Hz. Finally, all the spectral data are compatible with QCN. In ^{1}H-NMR, downfield signal at δ 9.05 (d, $J = 4.5$) is due to the proton at C-2 and coupling to C-3 proton. The other downfield signals centered at δ 8.22 (dd, 2H) are due to protons at C-5 and C-8. While the ddd at δ 7.88 is due to C-7, C-5 and C-8 protons, similarly ddd at δ 7.79 is due to C-6, C-8 and C-5 protons and d at δ 7.77 is due to C-2 proton. ^{1}H-^{1}H-COSY indicated the proton-proton coupling of δ 9.05 (d, H-2) with δ 7.77 (d, H-3); δ 8.22 (d, H-5) with δ 7.88 (ddd, H-6); δ 7.79 (ddd, H-7) with δ 7.88 (ddd, H-6); δ 7.79 (ddd, H-7) with δ 8.20 (d, H-8) (Figure S15). Similarly, ^{1}H-^{13}C-HSQC gave the proton coupling with corresponding carbons viz. δ 9.05 (d) with δ 149.4 (C-2); δ 8.22 (d) with δ 131.2 (C-5); δ 8.20 (d) with δ 130.4 (C-8); δ 7.88 (ddd) with δ 129.2 (C-6); δ 7.79 (ddd) with δ 124.9 (C-7); δ 7.77 (d) with δ 124.8 (C-3) (Figure S17). Finally, HMBC coupling as follows viz. H-2 (δ 9.05) with C-10 (δ 148.1), C-3 (δ 124.8), C-4 (δ 118.7); H-3 (δ 7.77) with C-2 (δ 149.4), C-11 (δ 115.5); H-5 (δ 8.22) with C-10 (δ 148.1), C-7 (δ 129.2), C-4 (δ 118.7); H-6 (δ 7.88) with C-8 (δ 130.4), C-9 (δ 125.8); H-7 (δ 7.79) with C-10 (δ 148.1), C-5 (δ 124.9); H-8 (δ 8.20) with C-6 (δ 131.2), C-9 (δ 125.8) (Figure S18). Thus, the isolated compound was identified as QCN.

2.4. Confirmation of compound through synthesis

The yield of QCN from quinoline-4-carboxaldehyde (2 g) was 1.4 g with m.p. 97 °C (Figure S19). Synthesized compound was identical as isolated QCN with respect to m. p. and super-imposable FT-IR, NMR and HR-MS data. Further, reduced products are

obtained at different temperature and solvent systems using heterogeneous catalysts (Table S3). These modified structures might be suitable for high value fragrance or pharmaceutical applications. In particular, quinoline-4-methylamine (QCM) was taken for the anti-inflammatory and antioxidant studies due to its different functional entity along with aromatic character as compared to the natural product.

2.5. Anti-inflammatory and antioxidant studies

Essential oil, absolute, QCN and QCM were screened for *in-vitro* anti-inflammatory activity against LPS-induced inflammation in macrophages isolated from peritoneal cavity of mice (Yang et al. 2018). Productions of pro-inflammatory cytokines (TNF-α, IL-1β) were quantified from cell culture supernatant using enzyme-linked immunesorbent assay (ELISA). Pro-inflammatory cytokines (TNF-α and IL-1β) was significantly ($p < 0.05$) increased in vehicle treated LPS-stimulated cells when compared with normal un-stimulated cells. The treatment of (Essential oil, absolute, QCN and QCM) has shown significant anti-inflammatory activity by inhibiting LPS-induced pro-inflammatory cytokines production in a dose-dependent manner as compared to vehicle treated LPS-stimulated cells. Percent inhibition of pro-inflammatory cytokines treatment is depicted in Table S4. Among the treatment, QCM possessed significant inhibition of TNF-α, IL-1β in a dose dependent manner followed by QCN, essential oil and absolute. The absolute was contained 19.7% of QCN along with other terpenoids and benzenoids. Therefore, absolute has been shown enhanced activity with lesser dose with respect to QCN. Interestingly, essential oil has been shown inferior activity, which might be correlated with least percentage of QCN (1.1%). Finally, anti-inflammation activity is depended on the QCN in the extract.

Antioxidant study revealed that the extracts have potent activities as presented in Table S5. Absolute has shown better antioxidant activity as compared to the isolated QCN. Enhancing antioxidant activity of absolute may be due to the phenolics as benzyl tiglate, (*Z*)-3-hexenyl benzoate, hexyl benzoate, phenyl ethyl tiglate, (*Z*)-3-hexenyl phenyl acetate, benzyl benzoate, etc. It has been inferred that the enhanced antioxidant activity of absolute due to the synergistic interaction among phenolics (10.1%) and QCN (19.7%). Polyphenols have played the synergistic role for enhancing antioxidant activities (Afify and Hassan 2016). Alternatively, QCM has been shown enhanced antioxidant activity as compared to QCN. Similarly, poor percentage of phenolics (8.3%) and QCN (1.1%) were responsible for inferior activity of the essential oil.

3. Conclusions

Essential oil is not true representative of volatiles emitted by flowers. Application of HS-SPME analysis gives scope for formulation through blending, which might be closely representing the floral fragrance. Similarly, live flowers analysis also provides scope to identify the chemical markers (particular composition) for attracting pollinators. QCN (5.8%) is a major compound in the live flowers of *Q. indica*, which is responsible for refreshing environment especially during evenings due to the presence of

this volatile alkaloid. Bio-efficacy study of this compound has been shown potent anti-inflammatory activity. Similarly, absolute contained 19.7% of QCN along with other terpenoids and benzenoids, which are in combination, revealed enhanced anti-inflammatory and antioxidant activities. This work is a complete characterization of natural product (QCN) with its bioactivity. Besides, heterogeneous catalytic reduction had produced QCM, which was shown enhanced anti-inflammatory and antioxidant activities.

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References

Afify A, Hassan H. 2016. Free radical scavenging activity of three different flowers-*Hibiscus rosa-sinensis*, *Quisqualis indica* and *Senna surattensis*. Asian Pac J Trop Biomed. 6(9): 771–777.

Anon. 1991. The wealth of India, publication & information directorate. New Delhi: CSIR, 5:370.

Lawrence BM. 1995. Isolation of aromatic materials from natural plant products. In: De Silva KT, editors. A manual on essential oil industry. Vienna, Austria: UNDO; pp. 60–74.

Mookherjee BD, Trenkle RW, Wilson RA. 1990. The chemistry of flowers, fruits and spices: live vs dead a new dimension in fragrance research. Pure Appl Chem. 62(7):1357–1364.

Pongcharoen W, Rukachaisirikul V, Isaka M, Sriklung K. 2007. Cytotoxic metabolites from the wood-decayed fungus xylaria sp. BCC9653. Chem Pharm Bull. 55(11):1647–1648.

Rout PK, Naik SN, Rao YR. 2008. Subcritical CO_2 extraction of floral fragrance from *Quisqualis indica*. J Supercrit Fluid. 45(2):200–205.

Xu L, Liu H, Ma Y, Wu C, Li R, Chao Z. 2018. Comparative study of volatile components from male and female flower buds of *Populus* X *tomentosa* by HS-SPME-GC-MS. Nat Prod Res. 33: 2105–2108.

Yan J, Wang M, Zhang L. 2018. Light induces petal color change in *Quisqualis indica* (*Combretaceae*). Plant Divers. 40(1):28–34.

Yan J, Wang G, Sui Y, Wang M, Zhang L. 2016. Pollinator responses to floral colour change, nectar, and scent promote reproductive fitness in *Quisqualis indica* (*Combretaceae*). Sci Rep. 6: 1-10. doi:10.1038/srep24408.

Yang W, Chen M, Meng D, Wei P. 2018. Potential anti-inflammation nor-oleanane triterpenoids from the fruits of *Stauntonia brachyanthera*. Nat Prod Res. pp.1-7. doi:10.1080/14786419.2018.1475388.AQ6