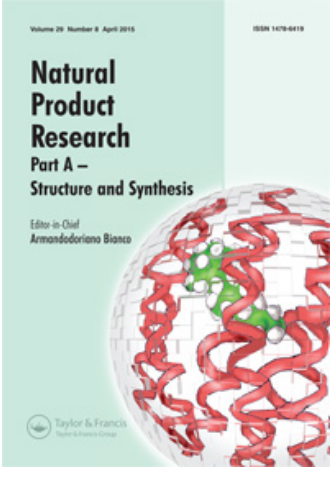




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# Identification and determination of flavonoids, carotenoids and chlorophyll concentration in Cynodon dactylon (L.) by HPLC analysis

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## SHORT COMMUNICATION

# Identification and determination of flavonoids, carotenoids and chlorophyll concentration in *Cynodon dactylon* (L.) by HPLC analysis

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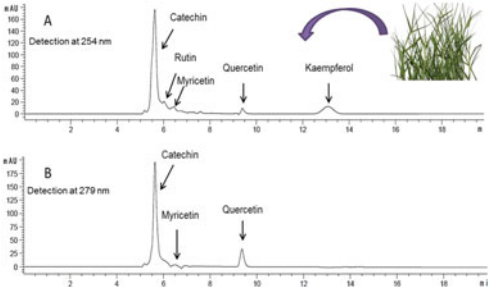


*Cynodon dactylon* (L.) is a potent medicinal plant in the traditional and current Indian medicinal systems. The objective of this research was to find out the levels of flavonoids, carotenoids and chlorophyll *b* in *C. dactylon* leaves by high-performance liquid chromatography (HPLC) equipped with a diode array detector. HPLC analysis revealed that total carotenoid and total flavonoid concentration were 62 mg/100 g and 249.1 μg/g, respectively. The mean chlorophyll *b* was 85.1 mg/100 g in *C. dactylon*. Among the flavonoids, quercetin (164.7 μg/g) was the major flavonoid followed by kaempferol (48.2 μg/g), rutin (18.4 μg/g), catechin (12.1 μg/g) and myricetin (5.7 μg/g). Of the carotenoids, β-carotene (35.2 mg/100 g) was predominant followed by lutein (17.0 mg/100 g), violaxanthin (5.8 mg/100 g) and zeaxanthin (4.2 mg/100 g). Chlorophyll *b* concentration was 85.1 mg/100 g in *C. dactylon*. The results of this investigation should be useful information for further pharmacological studies.

**Keywords:** *Cynodon dactylon*; carotenoids; chlorophyll *b*; flavonoids; HPLC

## 1. Introduction

Flavonoids are most ubiquitous groups of plant phenolics and every group of flavonoids has a capacity to act as antioxidants. Among them, flavones and catechin components act as the most powerful flavonoids for protecting the body against ROS (De Groot 1994). The other flavonoid components such as quercetin, kaempferol, myricetin and rutin have antioxidant, anti-inflammatory, antiviral and antiallergic, as well as anticancer activities (Fraga et al. 1987;

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Hillwell 1994). Carotenoids are natural pigments synthesised from plants, but these are not synthesised in humans, who therefore depend on dietary carotenoid sources (Fraser & Bramley 2004). Fruits and vegetables are the most important source of carotenoids in the human diet (Khachik et al. 1989). Of the various carotenoids, two major carotenoids in the human macula and retina are lutein and zeaxanthin, which help prevent age-related macular degeneration (Meydani et al. 1994; Olmedilla et al. 2001). Plant-derived β-carotene and β-cryptoxanthin are metabolised to produce vitamin A (Ziegler 1989), which plays a key role in vision, reproduction, bone growth, cell division and cell differentiation in humans (Stephens et al. 1996).

*Cynodon dactylon* is commonly known as Arugaum pullu and Doob in the Tamil and Hindi languages, respectively (Ashokkumar et al. 2013). It is a weed and has been found to possess various medicinal properties (Singh et al. 2009). The ethyl acetate extract of *C. dactylon* leaves has been reported to have anti-diabetic, antioxidant, immunomodulatory and anti-inflammatory activities (Saradha Devi et al. 2011). Several metabolites have been identified and quantified from aerial parts of *C. dactylon*, and it contains metabolites such as terpenoids, alkaloids, palmitic acid, phenolics, flavonoids and carotenoids (Johnson et al. 2002; Kopsell et al. 2010; Solanki & Nagori 2012; Annapurna et al. 2013; Bagewadi et al. 2014). High-performance liquid chromatography (HPLC) profiling of flavonoids from *C. dactylon* leaves was limited, and to our knowledge, this study was the first report for nutritionally important carotenoid identification and quantification in *C. dactylon* leaves from India. Therefore, the aim of this study was to find an appropriate flavonoid and carotenoid extraction protocol and to determine the level of flavonoid and carotenoid concentration from *C. dactylon* leaves. The obtained results will be important as an indication of *C. dactylon* leaves as a new source of bioactive flavonoids and carotenoids.

## 2. Results and discussion

### 2.1. *Range of linearity, and accuracy*

The selected standards of flavonoids, carotenoids and chlorophyll *b* and their molar mass (g/mol), molecular formula and purity (%) were presented (Supplementary Table S1; Supplementary Figure S1, online only). The linearity was investigated for the authenticated five flavonoids, four carotenoids and chlorophyll *b* standards by plotting the peak area against the injected amounts, and good correlation of linearity has been achieved. Retention time, regression equation and correlation coefficient determined from the standards are presented (Table 1). The accuracy of the analytical method was determined by assaying at least to triplicate the analysis of each sample. The recovery test was used to evaluate the accuracy of the method. Average recovery of flavonoids and carotenoids and relative standard deviation values are presented in Table 1. Flavonoid standards peaks were simultaneously identified using UV–vis diode array detection at 350 nm for kaempferol, 279 nm for catechin, myricetin and quercetin and 254 nm for rutin. All individual carotenoids and chlorophyll *b* were detected at 450 nm.

### 2.2. *Identification of flavonoids, carotenoids and chlorophyll* b

Three biologically replicated samples of *C. dactlylon* leaves were collected from the contaminant free area of the Avinashilingam University for Women, Coimbatore. The concentration of flavonoids and carotenoids was determined by regression equation (Table 1). *C. dactylon* leaves were separately extracted as ethyl acetate extract and methanol–dichloromethane extract was used for identification of flavonoids and carotenoids, respectively. The flavonoids catechin, rutin, quercetin, myricetin and kaempferol, and carotenoids violaxanthin, lutein, zeaxanthin and β-carotene for chlorophyll and chlorophyll *b* were identified in *C. dactylon* leaves. Furthermore, several unidentifiable peaks were also detected in

Table 1. Regression analysis data of flavonoids, carotenoids and chlorophyll of the leaf extract of *C. dactylon* by RP-HPLC.

| Sl no. | Compounds | RT[a] (minutes) | Lambda max[b] | Regression equation | $R^2$ | Recovery (%)[c] | RSD (%)[d] |
|---|---|---|---|---|---|---|---|
| *Flavonoids* | | | | | | | |
| 1. | Catechin | 5.81 | 279 | $7.9341x - 16.249$ | 0.9997 | 98.7 | 2.79 |
| 2. | Rutin | 6.02 | 254 | $4.3212x - 0.8010$ | 0.9999 | 98.5 | 2.52 |
| 3. | Quercetin | 9.45 | 279 | $5.7665x - 1.2141$ | 0.9995 | 99.1 | 0.82 |
| 4. | Myricetin | 6.22 | 279 | $6.2031x - 1.0151$ | 0.9998 | 99.2 | 1.41 |
| 5. | Kaempferol | 12.6 | 279 | $5.3746x + 0.1720$ | 0.9999 | 99.5 | 2.25 |
| *Carotenoids* | | | | | | | |
| 1. | Violaxanthin | 6.02 | 450 | $14.741x - 7.6619$ | 0.9978 | 99.9 | 3.14 |
| 2. | Lutein | 9.89 | 450 | $12.356x + 18.721$ | 0.9982 | 100 | 1.20 |
| 3. | Zeaxanthin | 11.2 | 450 | $12.605x - 12.216$ | 0.9985 | 99.9 | 3.25 |
| 4. | β-Carotene | 37.0 | 450 | $10.301x + 34.551$ | 0.9997 | 99.7 | 2.17 |
| *Chlorophyll* | | | | | | | |
| 1. | Chlorophyll *b* | 9.43 | 450 | $0.5505x - 6.2546$ | 0.9999 | 99.8 | 2.45 |

[a] Retention time.
[b] Lambda max, absorbance spectrum wavelength (nanometre).
[c] Average recovery ($n = 3$).
[d] RSD (%), relative standard deviation (%).

the chromatograms of both flavonoids and carotenoids, but these were not quantified due to only being present in minor concentrations and having unknown health benefits. Total flavonoid and total carotenoid concentrations were calculated as the sum of mean values of the five individual flavonoids and sum of mean values of four individual carotenoids, respectively. However, previous studies reported that phytoconstituents of different flavonoids, carotenoids (violaxanthin, β-carotene, lutein and zeaxanthin) and chlorophyll *b* were observed in *C. dactylon* (Johnson et al. 2002; Kopsell et al. 2010; Annapurna et al. 2013), and those results were confirmed in the present study results.

### 2.3. *Determination of flavonoids concentration*

Mean concentration of individual and total flavonoids is shown in Table 2. A typical sample chromatogram of identified flavonoids from leaves of *C. dactylon* is also presented (Supplementary Figure S2, online only). Ethyl acetate extract of *C. dactylon* leaves showed the highest concentration of quercetin followed by kaempferol (48.2 μg/g), rutin (18.4 μg/g), catechin (12.1 μg/g) and myricetin (5.7 μg/g). An observed concentration of quercetin (164.7 μg/g) was equal to that previously reported in *C. dactylon* (Jananie et al. 2011). Mean total flavonoid concentration (249.1 μg/g) was greater than the total flavonoid (170 μg/g) level observed in *C. dactylon* (Jananie et al. 2011). This higher total flavonoid concentration was probably due to the excellent sample extraction methodology used in our present study. Among the five flavonoids, quercetin and kaempferol are most abundant flavonoids in *C. dactylon.* In the present study, quercetin alone comprised the 66% of the total flavonoid concentration. Furthermore, kaempferol, rutin, catechin and myricetin comprised 19%, 7%, 5% and 2%, respectively, of the total flavonoids (Table 2).

### 2.4. *Determination of carotenoids and chlorophyll concentration*

Mean concentration of all carotenoids and chlorophyll *b* are presented in Table 2. Methanol–dichloromethane extract of *C. dactylon* had lower volatility and more than 99.7% recoveries for all individual carotenoids and chlorophyll *b*. The results revealed that methanol–dichloromethane extract of *C. dactylon* leaves predominantly showed β-carotene, followed by lutein, violaxanthin and zeaxanthin (Table 2). Mean zeaxanthin (4.2 mg/100 g) and lutein (17 mg/100 g) were within the level of previously reported zeaxanthin (3.0 mg/100 g) and lutein

Table 2. Compounds identified in the leaves of *C. dactylon* by RP-HPLC.

| Sl no. | Flavonoids | μg/g[a] ± SE[b] | % Comprising[c] | Carotenoids/ chlorophyll | mg/100 g[d] ± SE[b] | % Comprising[c] |
|---|---|---|---|---|---|---|
| 1 | Catechin | 12.1 ± 0.17 | 5 | Violaxanthin | 5.8 ± 0.23 | 9 |
| 2. | Rutin | 18.4 ± 0.26 | 7 | Lutein | 17.0 ± 0.33 | 27 |
| 3. | Myricetin | 05.7 ± 0.11 | 2 | Zeaxanthin | 4.2 ± 0.09 | 7 |
| 4. | Quercetin | 164.7 ± 1.58 | 66 | β-Carotene | 35.2 ± 0.72 | 56 |
| 5. | Kaempferol | 48.2 ± 0.30 | 19 | Total Carotenoids[e] | 62.0 ± 2.10 | 100 |
| 6. | Total flavonoids[f] | 249.1 ± 1.45 | 100 | Chlorophyll *b* | 85.1 ± 2.88 | – |

[a] Microgram per gram.
[b] Standard error ($n = 3$).
[c] Percent comprising the individual carotenoids and flavonoids with respective total concentrations.
[d] Milligram per 100 g.
[e] Total carotenoid concentration was calculated by the sum of mean values of four individual carotenoids.
[f] Total flavonoid concentration was calculated by the sum of mean values of five individual flavonoids.

(20.2 mg/100 g) in *C. dactylon* (Kopsell et al. 2010). However, the present study revealed that *C. dactylon* was a richer source of β-carotene concentration (35.2 mg/100 g) than the β-carotene concentration reported in golden rice endosperm (0.16 mg/100 g) (Beyer et al. 2002) and green cotyledon pea seeds (0.1–0.2 mg/100 g) (Holasová et al. 2009). Chlorophyll *b* concentration 85.1 mg/100 g were greater than the previously reported 50 mg/100 g chlorophyll *b* in *C. dactylon* (Kopsell et al. 2010). Mean total carotenoid concentration was 62 mg/100 g. A typical sample chromatogram of carotenoids profile of *C. dactylon* is presented (Supplementary Figure S3, online only). The present study results were confirmed: nutritionally important carotenoid β-carotene and lutein comprising 83% of total carotenoids were within the range of previously reported β-carotene and lutein that comprising 70% of total carotenoids in *C. dactylon* (Kopsell et al. 2010).

## 3. Conclusion

In developing countries where fruits and vegetables are expensive in off seasons, *C. dactylon* would be a good source of dietary flavonoids and carotenoids. Furthermore, *C. dactylon* leaves mostly abounded in quercetin and kaempferol concentration greater than those reported for curry leaf. Therefore, the present study suggests that the accumulation of a greater concentration of bioactive components such as quercetin and kaempferol in *C. dactylon* leaves will provide more useful information for future pharmacological investigations. However, *C. dactylon* are rich in β-carotene concentration, greater than those reported in rice, wheat, pea, chickpea, cassava and potato. Hence, the consumption of *C. dactylon* could address the problem of vitamin A and age-related macular degeneration deficiencies in developing countries.

## Supplementary material

Experimental details relating to this article are available online, alongside Table S1 and Figures S1–S3.

## Note

1. Both authors contributed equally to this article.

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