

Regulation of secondary metabolite production in the two members of the Cucurbitaceae family

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Abstract. The two species of the *Cucurbitaceae* family: *Momordica charantia* var. subsp. *charantia* and *Momordica charantia* var. *muricata* have gained increasing importance over recent years for their impeccable role in human health. Both medicinally important species synthesise various biochemical compounds that possess significant roles in different physiological conditions. Analyzing different enzymes under stress conditions can provide insights into plant defence mechanisms. The synchronous activity of different enzymes contributes to the plant's general health. A comparative analysis was done of different parts of both plants to evaluate which part can have the most beneficial effects on an individual's health upon consumption. Further, we get an insight into the plant parts that are best fit for consumption to benefit from the medicinal properties of this species.

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1 Introduction

Momordica charantia L., which belongs to the family Cucurbitaceae, is composed of two main Indian varieties: Karela (*M. charantia L. var. charantia*) and Uchhe (*M. charantia L. var. muricata*) [1]. Karela is a pale green perennial fruit famous for its bitterness and long-standing recognized nutritional and medicinal value [2], while Uchhe is a smaller monoecious annual vine with non-hairy tendrils [3]. The two varieties share the presence of biologically active compounds such as triterpenes, steroids, alkaloids, proteins, lipids, and polyphenols which are mainly responsible for their anti-diabetic activity [4].

Their fruits are laden with heteropolysaccharides, peptides, terpenoids, flavonoids, and phenolic compounds, thus being considered very nutritive among the Cucurbitaceae family [5]. Chlorogenic acid (CGA), the predominant phenylpropanoid, confers powerful antioxidant properties [6], whereas the skin of the fruit containing anthocyanins further increases functional value of the product [7]. A comparative analysis was done from different parts of both plants to evaluate which part can have the most beneficial effects on an individual's health on consumption. The results obtained from the different experiments suggest the antioxidant properties of the aforementioned species, in addition to their well-known antidiabetic properties. Further, we get an insight into the plant parts that are best fit

for consumption to benefit from the medicinal properties of this species.

2 Materials and methods

Our working samples consisted of the following:

Momordica charantia var. charantia – Species A

Momordica charantia var. muricata – Species B

2.1 Plant extract preparation:

The plant material is extracted following standard protocol [8]. With minor adjustments to a few parameters, the plant material is extracted as described below (Brolis et al. 1998).

2.2 Estimation of total chlorophyll:

The method of Lichtenthaler (1987) was utilized to measure the amount of chlorophyll with a few minor modifications. To finely homogenize 500 mg of leaf tissue, 100mL acetones were used. The homogenized sample was centrifuged at 12000 rpm for 15 minutes. Absorbance is measured to quantify chlorophyll levels.

2.3 Estimation of the extent of Lipid peroxidation:

With a few changes, the procedure from (Heath and Packer 1968) was used to estimate lipid peroxidation. Along with root tissues, 500 mg of shoots from leaves and petioles were collected. These were thoroughly homogenized in 4 mL of trichloroacetic acid and thiobarbituric acid [0.5% (w/v) thiobarbituric acid (SRL, Mumbai, India) in 20% (w/v) d trichloroacetic acid (TCA) (SRL, Mumbai, India). The solution was centrifuged for 10 minutes at 15,000 rpm.

Before measuring absorbance at 532 nm, the solution was boiled for 40 minutes and cooled for an hour at room temperature.

2.4 Phenolic estimation

2.4.1 Calculating the overall amount of polyphenols:

To calculate the overall polyphenol content (Talukder et al. 2016), some changes were made to the methodology of Singleton et al. (1999). Total polyphenols were measured by the Folin–Ciocalteu method [8]. Absorbance was taken at 760 nm after 45-minute dark incubation.

2.4.2 Calculating the overall amount of flavonoids:

The aluminium trichloride method was used to determine the total flavonoid content (Lin and Tang 2007) with some modifications (Talukder et al. 2016). Absorbance was recorded at 415 nm and compared with a rutin standard curve.

2.4.3 Calculating the overall amount of antioxidants:

To assess the overall antioxidant activity of phenolic compounds, the phosphomolybdenum assay (Prieto et al. 1999) was carried out with a few minor modifications (Talukder et al. 2016) [11].

2.4.4 Analysis using High Performance Liquid Chromatography (HPLC)

This HPLC procedure was carried out using a system with a HITACHI UV-VIS (U-2800, Tokyo, Japan) detector. Acetonitrile (Solution A) and 0.5% phosphoric acid solution (Solution B) (11.5:1) (88.5 VND/v) were used as the mobile phase. The flow rate was set at 1

ml/min and the injection volume at 20 μ l along with analysis of each sample for 20 min. The temperature in the column was kept constant at 252°C. From the calibration curve, the chlorogenic acid (99% pure, HPLC grade) concentrations in the various samples were ascertained. The standard curve constructed from the chromatogram is the peak area versus the standard concentration. To detect chlorogenic acid, the detector is set to a particular wavelength of 325 nm. Quantification was done by comparing the UV-vis spectra and retention periods of the samples to a reference chlorogenic acid. Spinchrom CFR software for HPLC and peaks was used to analyse the data. Peak areas were calculated in mV and mV x s, respectively (Dao and Friedman 1992)[1].

2.5 Gene expression analysis by RT PCR

2.5.1 Statistical analysis

The data is shown as mean \pm standard error. Significance thresholds: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)

2.5.2 Primer designing:

Primer designing for *HQT*, *ACTIN* gene - To create primers, published sequences from the NCBI database. Using the primer3 software (version 4.0), specific primers were created from conserved regions.

3 Results

3.1 Plant polyphenol estimation



Fig. 1. Total Polyphenol content of Seed, Skin and Pulp of Species A and Species B, respectively.

In Species A, the total polyphenol content was recorded as highest in the pulp (5807.10 GAE ($\mu\text{g/g}$) FW), whereas in skin, the recorded total polyphenol content was significantly lower than that of pulp (3035.70 GAE ($\mu\text{g/g}$) FW). The seed of the plant was considered as the control, and it recorded a total polyphenol content of 1521.40 GAE ($\mu\text{g/g}$) FW.

An identical pattern was seen in Species B in which the total polyphenol content was recorded highest in the pulp (6259.80 GAE ($\mu\text{g/g}$) FW), whereas in skin the recorded total polyphenol content was significantly lesser than that of pulp (2921.40 GAE ($\mu\text{g/g}$) FW). The seed of the plant was considered as the control, and it recorded a total polyphenol content of 2264.20 GAE ($\mu\text{g/g}$) FW.

3.2 Plant flavonoid estimation

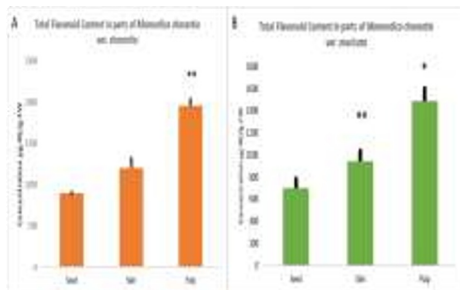


Fig. 2. Total Flavonoid content of Seed, Skin, and Pulp of Species A and Species B, respectively.

In Species A, the plant flavonoid estimation was recorded highest in the pulp (1958.333 $\mu\text{g-RE/g-FW}$), whereas in skin, the recorded total flavonoid content was significantly lower than that of pulp (1198.667 $\mu\text{g-RE/g-FW}$). The seed of the plant was considered as the control, and it recorded a total flavonoid content of 894 $\mu\text{g-RE/g-FW}$.

An identical pattern was seen in Species B, in which the total flavonoid content was recorded highest in the pulp (1490.667 $\mu\text{g-RE/g-FW}$), whereas in skin, the recorded total flavonoid content was significantly lower than that of pulp (944.333 $\mu\text{g-RE/g-FW}$). The seed of the plant was considered as the control, and it recorded a total flavonoid content of 701 $\mu\text{g-RE/g-FW}$.

3.3 Plant antioxidant estimation

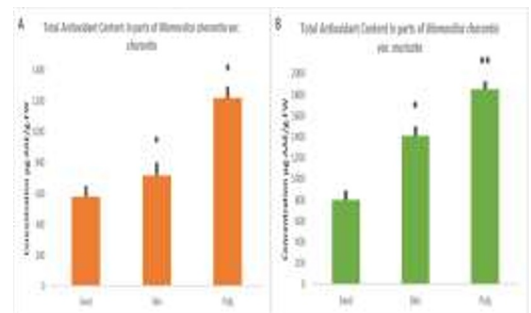


Fig 3. Total Antioxidant Content of Seed, Skin, and Pulp of Species A and Species B.

In Species A the total antioxidant content was recorded highest in the pulp (1220 $\mu\text{g-AAE/g-FW}$), whereas in skin the recorded total antioxidant content was significantly lesser than that of pulp

(717.333 $\mu\text{g-AAE/g-FW}$). The seed of the plant was considered as the control, and it recorded a total antioxidant content of 577.333 $\mu\text{g-AAE/g-FW}$.

An identical pattern was seen in Species B in which the total antioxidant content was recorded highest in the pulp (1849.333 $\mu\text{g-AAE/g-FW}$), whereas in skin the recorded total antioxidant content was significantly lesser than that of pulp (1410.667 $\mu\text{g-AAE/g-FW}$). The seed of the plant was considered as the control, and it recorded a total antioxidant content of 802.333 $\mu\text{g-AAE/g-FW}$.

3.4 Estimation of MDA content by lipid peroxidation assay

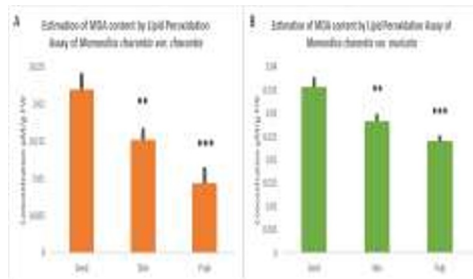


Fig 4. Total Malonaldehyde Content of Seed, Skin, and Pulp of Species A and Species B, respectively.

In Species A, the total MDA content was recorded highest in the skin (0.015167 $\mu\text{M/g-FW}$), whereas in pulp, the recorded total MDA content was significantly lower than that of the skin (0.0093 $\mu\text{M/g-FW}$). The seed of the plant was considered as the control, and it recorded a total MDA content of 0.022 $\mu\text{M/g-FW}$.

An identical pattern was seen in Species B in which the total MDA content was recorded highest in the skin (0.0283 $\mu\text{M/g-FW}$), whereas in pulp the recorded total MDA content was significantly lesser than that of skin (0.024 $\mu\text{M/g-FW}$). The seed of the plant was considered as the control, and it recorded a total MDA content of 0.03567 $\mu\text{M/g-FW}$.

3.5 Hydroxycinnamoyl Co-A Quinate Transferase (HQT) gene expression analysis

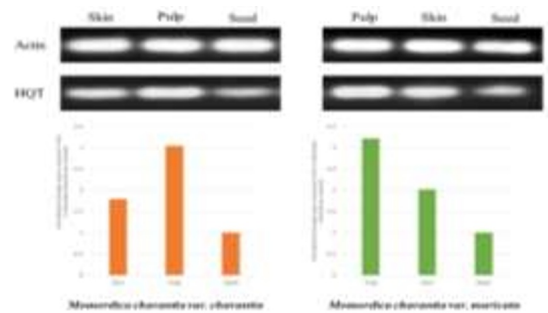


Fig. 5. Illustrations of Gel electrophoresis and graphical data showing the fold change in the HQT gene expression levels in Species A and Species B, respectively.

In the case of Species A, we observe the highest expression of the HQT gene in the pulp. The expression of HQT found in the skin is slightly lower than in the pulp, followed by a considerable decline in the gene expression in the seed. In comparison to the seed, the skin shows a 0.7 times higher fold change. Whereas, the pulp depicts nearly 2 times higher fold change than the seed.

A similar trend is observed in Species B, where the pulp shows the highest HQT gene expression. HQT gene expression is

somewhat lower in the skin than in the pulp, and then it significantly declines in the seed. The skin exhibits a 1.0 times greater fold change than the seed. However, the pulp shows a nearly 3.2-times higher fold change compared to the seed. In both cases, the seed shows the lowest gene expression.

3.6 Chlorogenic acid concentration

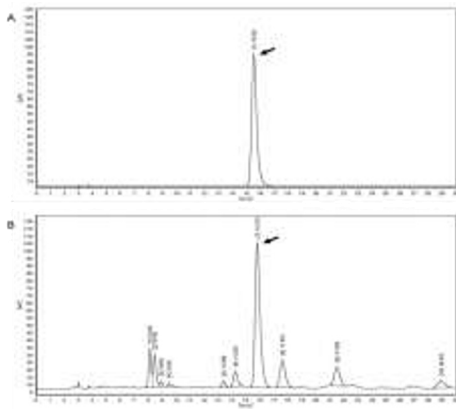


Fig 6. Chromatogram of HPLC analysis (A) Chlorogenic Acid (CGA) standard (B) 80% ethanolic extract of Species B pulp

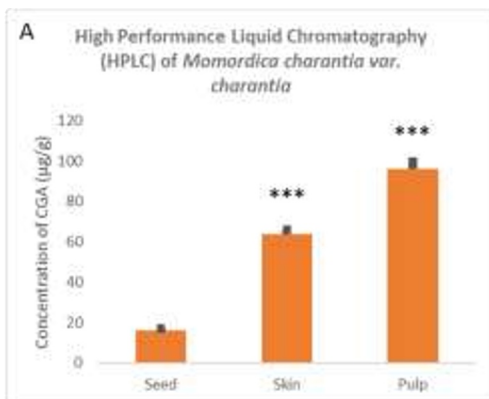


Fig 7. Concentration of Chlorogenic Acid by HPLC analysis in Seed, Skin, Pulp of Species A and Species B.

In Species A, the highest levels of CGA concentration were found in the pulp (73.133 µg/g), followed by a significant decrease in the case of skin (43.567 µg/g). The seed was taken as the control, and the CGA content was recorded at a value of 28.6 µg/g. An analogous orientation was observed in Species B, where the CGA content was the highest in pulp (56.4667 µg/g) followed by skin (36.567 µg/g). The seed was taken as the control and the CGA content was recorded at a value of 22.533 µg/g (Table 2). The CGA content was found to be maximum in the pulp of Species A and much lower in Species B,

4 Discussion

Both Cucurbitaceae species, Species A and Species B, possess substantial medicinal value, particularly due to their hypoglycemic properties that help reduce blood glucose levels [9]. The present study shows that both species contain higher polyphenolic content in the edible pulp than in the skin tissues, although previous reports on Chinese and Indian *Momordica* varieties indicate significantly greater polyphenols in skin tissues compared to pulp and seeds [10]. In both species, flavonoid concentration is highest in the pulp. A related study on *M. charantia* demonstrated that methanolic extracts yield higher flavonoid content than chloroformic extracts [11].

Plants with elevated antioxidant content display strong medicinal and herbal potential [12]. The current findings reveal a similar trend, where antioxidant levels are higher in pulp tissues, aligning with earlier studies reporting greater antioxidant content in pulp than in skin

and seeds of *Momordica* species [10]. The phenylpropanoid pathway, initiated by phenylalanine ammonia-lyase (PAL), produces chlorogenic acid (CGA) along with flavonoids, coumarins, and lignans [13]. CGA has been validated as a major polyphenol in *Momordica* through HPLC studies [10], and additional polyphenols were detected in the present analysis [14]. HQT gene expression, essential for CGA synthesis, showed 2-fold higher pulp expression in Species A and 3.2-fold in Species B, with seeds showing the lowest expression in both species [15].

5 Conclusion

The study clarifies the important medical advantages of Cucurbitaceae species A and B. In this novel study, the antioxidant properties have been brought to light. Extensive phytochemical analyses demonstrated significant levels of chlorogenic acid (CGA) in the pulp, seeds, and skin of different fruit components. Interestingly, the pulp - a significant edible portion- showed the greatest concentrations of antioxidants, polyphenols, and flavonoids in both species. However, pulp tissue showed less total oxidative stress, which is consistent with earlier research showing a negative relationship among stress levels as well as metabolite stability. The pulp's high content of polyphenols and antioxidants not only demonstrates the fruit's ability to reduce inflammation but also bolsters its effectiveness in regulating blood sugar levels. These results open the door to more research into the therapeutic uses of members of the Cucurbitaceae family, which might result in the development of novel dietary approaches to the treatment of inflammatory diseases and diabetes.

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Author contribution statement: PT conceptualized, supervised and cross-checked the manuscript. All the authors contributed to the experiment, analysis and write-up of this work.

References

1. S.K. Sharma, P. Baliyan, A. Alam, *Momordica charantia* L.: Unlocking its Potential as a Nutritional Food Through Ethnomedicinal and Pharmacological Properties. *Res. J. Bot.* **19**, 31–40 (2024). <https://doi.org/10.3923/rjb.2024.31.40>
2. M.W. Mazhar, M. Ishtiaq, M. Maqbool, E.A. Mahmoud, F. Ullah, H.O. Elansary, Optimizing bitter gourd (*Momordica charantia* L.) performance: exploring the impact of varied seed priming durations and zinc oxide nanoparticle concentrations on germination, growth, phytochemical attributes, and agronomic outcomes. *Cogent Food Agric.* **10**, 2313052 (2024). <https://doi.org/10.1080/23311932.2024.2313052>
3. A. Suma, M.A. Alfia, K.J. John, K. Pradheep, G.D. Harish, P.P. Thirumalaisamy, M. Latha, On the development of descriptors in small bitter gourd (*Momordica charantia* L. var. *muricata* (Willd.) Chakrav). *Genet. Resour. Crop Evol.* **70**, 289–308 (2023). <https://doi.org/10.1007/s10722-022-01494-4>
4. Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J, Astatkie T. 2022. Applications of essential oils and plant extracts in different industries. *Molecules.* 27(24):8999. [doi: 10.3390/molecules27248999](https://doi.org/10.3390/molecules27248999)

5. Chaudhury P. 2023. Role of Secondary Metabolites of *Momordica charantia* in combating Copper induced stress. American Journal of Applied Bio-Technology Research. 4(1):5–29.
6. Cheng J, Chen Y, Guo F, Dong P, Zhou C, Liang W, Wang H. 2025. Regulatory mechanisms and biosynthesis of chlorogenic acid in *Lonicera japonica*: insights from tissue culture and inducer treatments. Frontiers in Plant Science. 16:1567140. doi:[10.3389/fpls.2025.1567140](https://doi.org/10.3389/fpls.2025.1567140)
7. D'Orso F, Hill L, Appelhagen I, Lawrenson T, Possenti M, Li J, Harwood W, Morelli G, Martin C. 2023. Exploring the metabolic and physiological roles of HQT in *S. lycopersicum* by gene editing. Frontiers in Plant Science. 14:1124959. doi:[10.3389/fpls.2023.1124959](https://doi.org/10.3389/fpls.2023.1124959)
8. Fatima MT, Bhat AA, Nisar S, Fakhro KA, Akil AS. 2023. The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: An assessment of the benefit profile. Heliyon. 9:e12398. doi: [10.1016/j.heliyon.2022.e12698](https://doi.org/10.1016/j.heliyon.2022.e12698)
9. Jayaraj MS, Beevy SS. 2021. Impact of drought on the characteristic's attributes in the varieties of *Momordica charantia* L. Int J Botany Stud. 6(3):125–131.
10. Kim B, Lee HS, Kim HJ, Lee H, Lee IY, Ock S, Kwon S, Kang SS, Choi Y. 2023. *Momordica charantia* (bitter melon) efficacy and safety on glucose metabolism in Korean prediabetes participants: a 12-week, randomized clinical study. Food Science and Biotechnology. 32(5):697–704. doi: [10.1007/s10068-022-01214-9](https://doi.org/10.1007/s10068-022-01214-9)
11. T. Kowalczyk, M. Muskała, A. Merez-Sadowska, J. Sikora, L. Picot, P. Sitarek, Anti-inflammatory and anticancer effects of anthocyanins in in vitro and in vivo studies. Antioxidants 13, 1143 (2024). <https://doi.org/10.3390/antiox13091143>
12. P. Talukder, S. Talapatra, N. Ghoshal, S. Sen Raychaudhuri, Antioxidant activity and HPLC analysis of phenolic compounds during in vitro callus culture of *Plantago ovata* Forsk. and effect of exogenous additives. J. Sci. Food Agric. 96, 232–244 (2016). <https://doi.org/10.1002/jsfa.7086>
13. Lv J, Yang S, Zhou W, Liu Z, Tan J, Wei M. 2024. Microbial regulation of plant secondary metabolites: Impact, mechanisms and prospects. Microbiological Research. 283:127688. doi: [10.1016/j.micres.2024.127688](https://doi.org/10.1016/j.micres.2024.127688)
14. Marčetić M, Arsenijević J. 2023. The Antioxidant activity of plant secondary metabolites. Arh Farm. 73:264–277. doi: [10.5937/arhfarm73-45560](https://doi.org/10.5937/arhfarm73-45560)
15. Nath PC, Dey P, Paul T, Shil S, Sarkar S, Rustagi S, Bhattacharya D, Vora K, Roy R. 2024. Essential oils and their critical implications in human use. Biocatalysis and Agricultural Biotechnology. 60:103258. doi: [10.1016/j.bcab.2024.103258](https://doi.org/10.1016/j.bcab.2024.103258)