



Phytochemical profile of onion (*Allium cepa*) var. *aggregatum*: Comparative study of TNAU varieties with a local indigenous

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ABSTRACT

Aggregatum onion (*Allium cepa* L. var. *aggregatum* Don.) is widely grown and consumed in southern India. Present study was carried during 2020–23 at Anbil Dharmalingam Agricultural College and Research Institute (Tamil Nadu Agricultural University), Tiruchirappalli, Tamil Nadu to map the metabolites and other dietary nutrients contributing to nutraceutical potentials between indigenous varieties and aggregatum onions varieties of Tamil Nadu. Experiment consisted of Co (On) 5 and Co (On) 6 (Tamil Nadu Agricultural University released varieties) and an indigenous variety from Perambalur (PL), Tamil Nadu. In the present study, metabolites of all the 3 varieties were mapped with the GCMS/MS, and the PL was shown to have high phytonutrients with health benefits. Further, in UHPLC, quercetin was identified with a sharp, well resolved symmetrical peak for all the three onion varieties with the retention time of 64.5, 65.5 and 64.5 min respectively. The Co (On) 6 is the improved variety of Co (On) 5 with a quercetin content of 75.05 ± 0.30 mg/kg, followed by PL (11.11 ± 0.51 mg/kg). However, the native cultivar PL (4.52 ± 0.15 mg/kg) has the highest anthocyanin content followed by Co (On) 6 (4.00 ± 0.10 mg/kg). This study therefore, provides preliminary information on metabolites and phenolics in the selected onion varieties of Tamil Nadu.

Keywords: Aggregatum, Anthocyanin, Co (On) 5, Co (On) 6, Quercetin

Onion (*Allium cepa* L.) is one of the cash crops cultivated under different climatic conditions and consumed worldwide. It is the world's second most produced vegetable after tomato. The bulb shape and size, scale colour, dry matter content, pungency, flavour and bulb storage durability may vary for different cultivars (Bottcher *et al.* 2017). It is not only used as a part of international cuisine (raw or cooked) but also used in traditional medicine. Regular consumption of onion can lower the risk of getting type 2 diabetes, cancer, cardiovascular diseases, cataract, viral infections and gastrointestinal tract infections. It also aids in liver function improvement and obesity prevention (Cozzolino *et al.* 2021).

The top onion producers around the world includes China, India, USA followed by Pakistan, Russia, Iran, Brazil, Mexico and Spain. In India, during 2020–2021, 22.8 million metric tonnes were recorded as annual production which was achieved from 1.22 million hectares

(Velmurugan *et al.* 2022). Onions are grown commercially in two varieties around the world. The common onion grows from seeds and has huge, solitary bulbs. Onion var. *aggregatum* Don. grows in massive numbers of tiny bulbs, forming an aggregated cluster, and is propagated by bulbs and seeds (Geethanjali and Lakshmanan 2021). The dried outer scales of aggregatum onion contain several phytochemicals with antioxidative and antibacterial activities (Ly *et al.* 2005).

As agriculture has modernized, farmers have increasingly focused on generating hybrids and abandoned open-pollinated genotypes and landraces, resulting in genetic loss. Landraces can help to preserve biodiversity, genetic diversity and metabolite diversity. Several pre and post-harvest parameters have been connected to long-term onion storage. Changes in metabolite concentrations are also possible during bulb curing and storage (Romo-Perez *et al.* 2019). Hence, the aim of this present study was to map the metabolites and other dietary nutrients contributing to nutraceutical potentials of aggregatum onion varieties of Tamil Nadu. Also, it's a virgin study where indigenous and varieties of aggregatum onion were compared with metabolites of GCMS/MS. The findings of this study will provide a comprehensive picture of the numerous metabolites and phytonutrients found in aggregatum onions,

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and this baseline research will be useful in post-harvest storage research in onions.

MATERIALS AND METHODS

Collection and preparation of samples: Present study was carried during 2020–23 at Anbil Dharmalingam Agricultural College and Research Institute (Tamil Nadu Agricultural University), Tiruchirappalli, Tamil Nadu, district known for its aggregatum onion farming. Indigenous and varieties of aggregatum onions were compared in this study. The aggregatum onion samples PL (indigenous), Co (On) 5 and Co (On) 6 (varieties) were collected from several farms in the Perambalur district. The obtained samples were shade dried and frozen with liquid nitrogen before being pulverized with a pestle and mortar to produce a fine homogeneous powder for biochemical analysis (Cecchi *et al.* 2020).

Phytochemical extraction and estimation: 250 mg of onion powdered samples were treated overnight in 10 ml of 70% ethanol to extract phytochemicals. Further it was centrifuged at 10000 rpm for 20 min and supernatant obtained was collected and subjected to biochemical analysis. The total phenolic contents of the extract were analyzed by Folin-Ciocalteu (FC) assay with gallic acid as the standard (Eberhardt *et al.* 2000, Sadasivam and Manickam 2004), and the concentrations were expressed in mg gallic acid equivalents (GAE, mg Gallic acid/kg). Total flavonoid content (TFC), was determined by Zhishen *et al.* (1999) method with minor changes with quercetin as the standard, results were represented as mg quercetin equivalence (mg QE/kg).

Metabolite screening by UHPLC and GCMS/MS: UHPLC and GCMS/MS were used to analyze polar and non-polar metabolites. 10 g of sample was homogenized with 25 ml of 100% methanol. Samples were vortexed for 5 min and kept in sonicator for 30 min followed by centrifugation at 4,500 rpm for 10 min. Filter the supernatant in 0.45 µm syringe filter. Then the samples were injected in HPLC system to analyze quercetin and anthocyanin.

To quantify and identify quercetin concentration in onion samples, an HPLC system (Agilent Technologies 1100 Series, USA) equipped with a quaternary pump, a degasser, a thermostatic auto-sampler, and a diode array detector (DAD) was employed. Quercetin was separated using Shimpack C18 column (4.6 mm × 250 mm, 5 µm particle size) at 30°C. The quercetin standard was dissolved in methanol. The detector was adjusted at 370 nm to measure quercetin. The thermostat in the sample was adjusted to 5°C, which enables accurate temperature control, and the sample was run for 90 min. The resulting chromatogram was checked for the existence of quercetin using the retention time of the samples with reference to the quercetin standard (Tsao and Yang 2003).

An HPLC system (Agilent 1260 Infinity II, USA) coupled with UV-DAD was used to quantify and characterize anthocyanin content in onion samples. At 20°C, anthocyanin was separated using a Pursue XRs C18 analytical column

(4.6 mm × 250 mm), with particle sizes of 5 µm. The binary mobile phase was consisted of 6% acetic acid in 2 mM sodium acetate (final pH 2.55, v/v, solvent A) and acetonitrile (solvent B). The detector was adjusted to 520 nm for anthocyanin detection. The sample was run for 10 min. The resulting chromatogram was checked for the presence of anthocyanin by comparing the retention time of the samples against the anthocyanin standard (Tsao and Yang 2003).

GCMS/MS conditions: The phytochemical screening of aggregatum onion sample was detected using the column Rtx-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30 m × 0.25 mm ID × 0.25 mm df, and detector Single Quadrupole Mass Spectrometer (Agilent, USA). The carrier gas flow rate was kept constant at 1 ml/min, and the split ratio was maintained at 10:1. The injection volume was 2 µl and the injector temperature was kept at 280°C. The NIST database was used to interpret the MS results, and the spectra of unknown substances were compared to known samples in the library database (Shani *et al.* 2014).

Statistical analysis: All analyses were performed in triplicate, and the results are given as mean ± standard deviation on a dry weight basis. Using SPSS statistics, the data is subjected to one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) (version 26).

RESULTS AND DISCUSSION

Changes in total phenol (TPC) and total flavonoid content (TFC): TPC and TFC in the onion varieties are depicted in Table 1. PL had the highest total phenol concentration (800.4 ± 31.72 mg GAE/kg DW), followed by Co (On) 6 (760.5 ± 17.81 mg GAE/kg DW). PL also has the greatest total flavonoid concentration (624.2 ± 13.49 mg QE/kg DW of onion). The total flavonoid content in Co (On) 6 and Co (On) 5 was 545.2 ± 15.24 and 525.4 ± 5.76 mg QE/kg DW respectively. Sidhu *et al.* (2019) analyzed the phenolic content of 6 major Kuwaiti onion cultivars, reporting total phenol levels ranging from 8.16 to 22.63 mg/g in outer scales, 6.19 to 16.35 mg/g in middle scales, and 3.69 to 15.75 mg/g in inner scales. Sagar *et al.* (2020) stated that the TPC for cv. Bhima Shubhra and NHRDF Red, ranged from 14.55 ± 0.41 mg to 288.74 ± 1.27 mg GAE/g. Similarly, the maximum and minimum TFC levels were found in cv. NHRDF Red at 168.77 ± 0.87 mg QE/g DW and Bhima Shubhra at 1.31 ± 0.32 mg QE/g DW, respectively.

The results show that the phenolic compounds differed between varieties, indicating that environmental factors such as day length, intensity of light, temperature, nutrient concentration, and soil water influence the synthesis of secondary metabolites, specifically phenolic and flavonoid constituents (Bibi *et al.* 2022). Also, onion chemical composition is influenced by cultivar, habitat, agronomic practices, maturation stage, and storage duration. Plants respond to a wide range of abiotic and biotic stresses, which weaken the plant and make it more prone to disease, reducing plant nutrition. Plants accumulate phenolic compounds that

serve as antioxidants, structural polymers, signal molecules, and defensive response antibacterial and antioxidants, supporting the plant in avoiding pathogenic infections and reactive oxygen species.

Changes in the quercetin content: The quercetin standard was used in this experiment, and the separation was done for 70 min in C18 reverse phase column. A sharp, well resolved symmetrical peak were observed for all the three [Co (On) 5, Co (On) 6 and PL type] onion samples with the retention time of 64.5, 65.5 and 64.5 min respectively. The chromatograms produced were qualitatively similar, which is consistent with earlier findings (Basava Prasad *et al.* 2022). The HPLC peaks labeled in Fig. 1 (A, B and C) are specified in Table 1.

The quercetin content of three onion cultivar ranged from 0.98 to 75.05 mg/kg DW of onion. Results showed that among the released cultivars of TNAU, the Co (On) 6 varieties had the highest quercetin content (75.05 ± 0.30 mg/kg) whereas, the Co (On) 5 exhibits lowest levels of quercetin (1 ± 0.00 mg/kg). However, the local onion germplasm showed higher quercetin content (11.11 ± 0.51 mg/kg) than Co (On) 5 variety. Recent research has shown that the quercetin concentration in different onion cultivars ranges from 74.91 to 34.67 mg/kg in onion cultivars produced from Punjab Agricultural University, Ludhiana, viz. Punjab Naroya, PRO-6, Punjab white, and Commercial (Majid *et al.* 2021). Another study by Kwak *et al.* (2017) observed quercetin concentrations ranging from 32.21 mg/g DW to 127.92 mg/g DW in three onion varieties. Quercetin is one of the dietary flavonoids with antioxidant properties. It has been proven to possess many health benefits, including protection against osteoporosis, lung cancer, and cardiovascular disease (Anand David *et al.* 2016).

Along with the quercetin peak, one major peak and three minor peaks were found in the quercetin chromatogram. Quercetin can be found either free or conjugated with carbohydrate, primarily as glycosides. The two main quercetin glycosides found in onions are quercetin-3,4'-O-diglucoside and quercetin-4'-O-monoglucoside, which account for about 80% of the total flavonol content (Sharma *et al.* 2014). Another study found five quercetin derivatives in onion var. *aggregatum* and reported that the quercetin concentration of outer dry skin was 83%, while inner tissues accounted for only 1% (Wiczowski *et al.* 2008).

Changes in anthocyanin content: Anthocyanins are phenolic compounds found in plants that are responsible for the purple, red colour of onion and are significantly concentrated in the skin (Metrani *et al.* 2020). Kuromanin chloride also identified as cyanidin-3-O-glucoside, is the chief form of anthocyanin that is found in most of the plants. A sharp, well-resolved, and proportional peak was identified in this analysis for three onion samples [Co (On) 5, Co (On) 6, and PL] with a retention time of 3.68 min at 520 nm and peak heights of 0.264, 0.436, and 0.632, respectively. Three aggregatum cultivars had anthocyanin contents ranging from 3.51 to 4.52 mg/kg. The PL cultivar had the highest anthocyanin concentration (4.52 ± 0.15), followed by Co (On) 6 (4.00 ± 0.10) and Co (On) 5 (3.51 ± 0.15). The HPLC peaks are labeled in Fig. 2 and Table 1.

Thus, the two primary flavonoids present in onions are quercetin and anthocyanin. The findings are similar to those reported by Vijayalashmi *et al.* (2021) who discovered 3.79 ± 0.06 and 3.41 ± 0.15 mg/kg of cyanidine O glucoside in onion and land race Krishnapuram bulb. Perez-Gregorio (2010) observed a similar rise in anthocyanin from outer to inner scales ranging from 5.15 to 1.24 mg/kg of cyanidin 3 glucoside in a local onion cultivar.

Anthocyanins are potent free radical scavengers that have been shown to protect against oxidative DNA cleavage, enzyme inhibition, oxidative lipid degradation, and membrane strengthening. They also promote cardiovascular health and the prevention of Alzheimer's and Parkinson's illnesses (Metrani *et al.* 2020). In general, when it comes to the health advantages of onions, red onion cultivars outperform white and yellow cultivars because they contain more antioxidants (quercetin and anthocyanin), which may be responsible for their cancer-prevention properties.

Phytochemical screening by GCMS: The detailed information on number of compounds eluted in different onion varieties are given in Table 2. The mass spectrum of the bioactive compounds with respective retention time (RT) is exhibited in Fig. 3 (A, B and C).

The metabolites eluted at varied retention times in Co (On) 6 and Co (On) 5 are 28 and 22, respectively, with 14 phytochemicals shared.

The above mentioned phytochemicals also include carboxylic acid esters, sugar derivatives, sulphur compounds and so on. The Co (On) 6 contains specific phytochemicals

Table 1 Quantification of phenolics

Variety	Total phenol (mg GAE/kg)	Total flavonoids (mg QE/kg)	Quercetin (mg/kg)	Anthocyanin (mg/kg)
Co (On) 5	$660.2 \pm 8.29^{b**}$	525.4 ± 5.76^b	1 ± 0.00^c	3.51 ± 0.15^c
Co (On) 6	760.5 ± 17.81^a	545.2 ± 15.24^b	75.05 ± 0.30^a	4.00 ± 0.10^b
Perambalur (Local)	800.4 ± 31.72^a	624.2 ± 13.49^a	11.11 ± 0.51^b	4.52 ± 0.15^a
S.E. (M)	12.44	7.05	0.19	0.07
C D ($P=0.05$)	43.89	24.88	0.70	0.28

**The one-way ANOVA and Duncan's Multiple Range Test (DMRT) revealed that the mean in each column preceded by the same letter were not significantly different ($P<0.05$). The values were the means of three replications $\pm$ standard deviation.

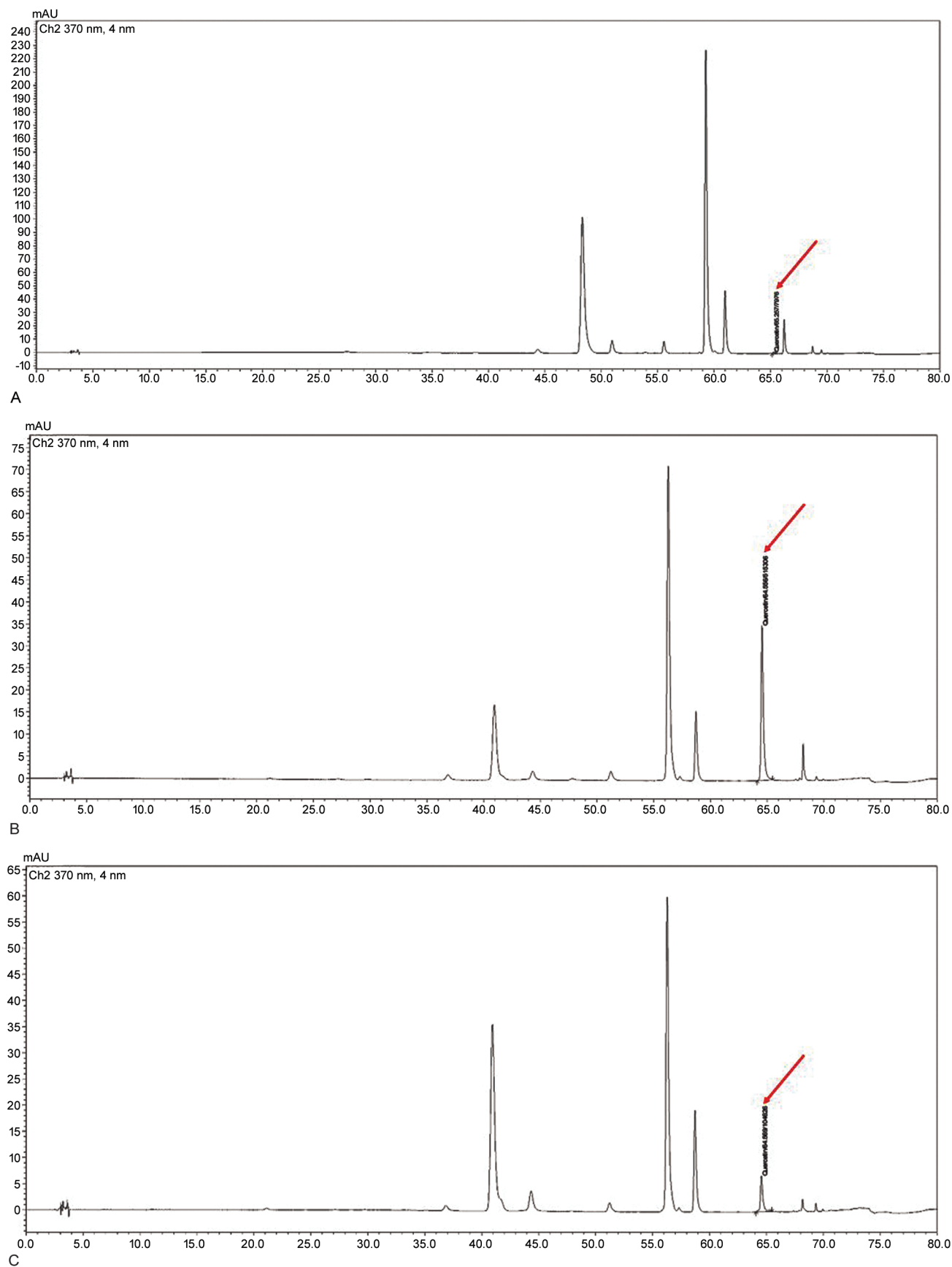


Fig. 1 UHPLC quercetin chromatogram (marked in red) pattern of different varieties of onion.

The quercetin standard was detected at 370 nm. Samples, (A) Co (On) 6; (B) Co (On) 5 (TNAU varieties); (C) Perambalur local (Local variety).

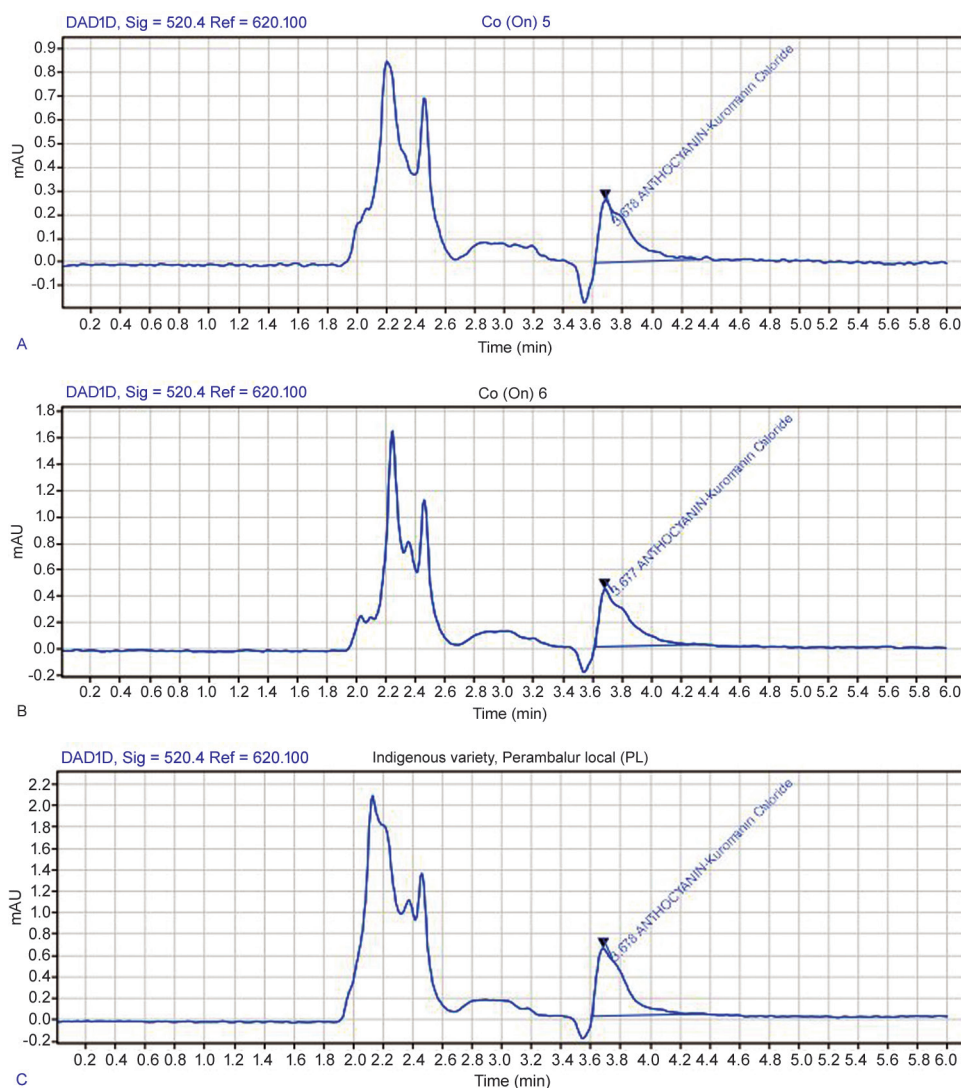


Fig. 2 UHPLC anthocyanin chromatogram pattern of different varieties of onion.

The anthocyanin standard was detected at 520 nm. Samples (A) Co (On) 6; (B) Co (On) 5 (TNAU varieties); (C) Perambalur local (Local variety).

Table 2 Number of compounds eluted in different onion varieties

Name of the biochemical analysis	Number of compounds		
	Co (On) 6	Co (On) 5	Perambalur local
GC MS/MS	28	22	37

like 9-Cyano-1-methyl-3, 6-diazahomoadamantan-9-ol which is also termed as Triacsin C, is a long chain fatty acyl CoA synthetase inhibitor that disrupts fat metabolism by preventing *de novo* production of glycerolipids and cholesteryl esters. It is clear that, onion is beneficial in lowering cholesterol levels. According to the Lu *et al.* (2015) drinking onion juice can significantly lower cholesterol levels and increases antioxidant property. A photochemical compound, Tetraacetyl-d-xylonic nitrile, is reported to have its pharmacological functions of anti-tumor and antioxidant is found in Co (On) 6 varieties. It also contains saturated, Mono and Poly unsaturated fatty acids, ketones and steroid compounds.

In local (Perambalur) variety, totally 37 phytonutrients were detected. In that, only 9 were found in common with the Co (On) 5 and Co (On) 6 and remaining, 28 compounds are different. The 5-(Hydroxymethyl) dihydrofuran-2(3H)-one is used for the production of mevinic acids, which reduce LDL cholesterol. It is obvious that naturally local onion cultivars inhibit the development of harmful cholesterol in the body. 1H-Azepine, hexahydro-1-nitroso-is also named as azepine, are antidepressants. It is an unsaturated heterocyclic compound used for controlling bipolar disorder, schizophrenia, and major depressive disorders. Opuntiol ($C_7H_8O_4$) is a substance used for treating diabetes, high cholesterol, obesity and hangovers. It is also publicized for its antiviral and anti-inflammatory properties. Campesterol is a plant-derived steroid with cholesterol-lowering characteristics and the potential to prevent cancer.

In addition, the local type onion is also found to contain, n-Hexadecanoic

acid; 9,12-Octadecadienoic acid (Z,Z); Octadecanoic acid; 9,12-Octadecadienoic acid (Z,Z); 2,3-dihydroxypropyl ester; 9-Octadecenoic acid (Z); tetradecyl ester and Hexadecanoic acid; 1-(hydroxymethyl)-1,2-ethanediylester. 9,12-Octadecadienoic acid (Z,Z) is an important unsaturated fatty acid found in animal diets that is needed for the synthesis of prostaglandins and cell membranes.

The aggregatum onion is a versatile vegetable that is used in a variety of recipes. It consists of several bioactive compounds which could serve as useful antioxidants for human health. This study looked at the phenolics and metabolites identified in aggregatum onion varieties such as Co (On) 5, Co (On) 6 and PL from Tamil Nadu. According to the UHPLC findings, TPC, TFC, and anthocyanin concentrations were greater in PL, whereas quercetin concentrations were higher in Co (On) 6, followed by PL. Similarly, a significant number of phytochemicals with health advantages were discovered in PL. To summarize, PL has a wide range of phytochemicals

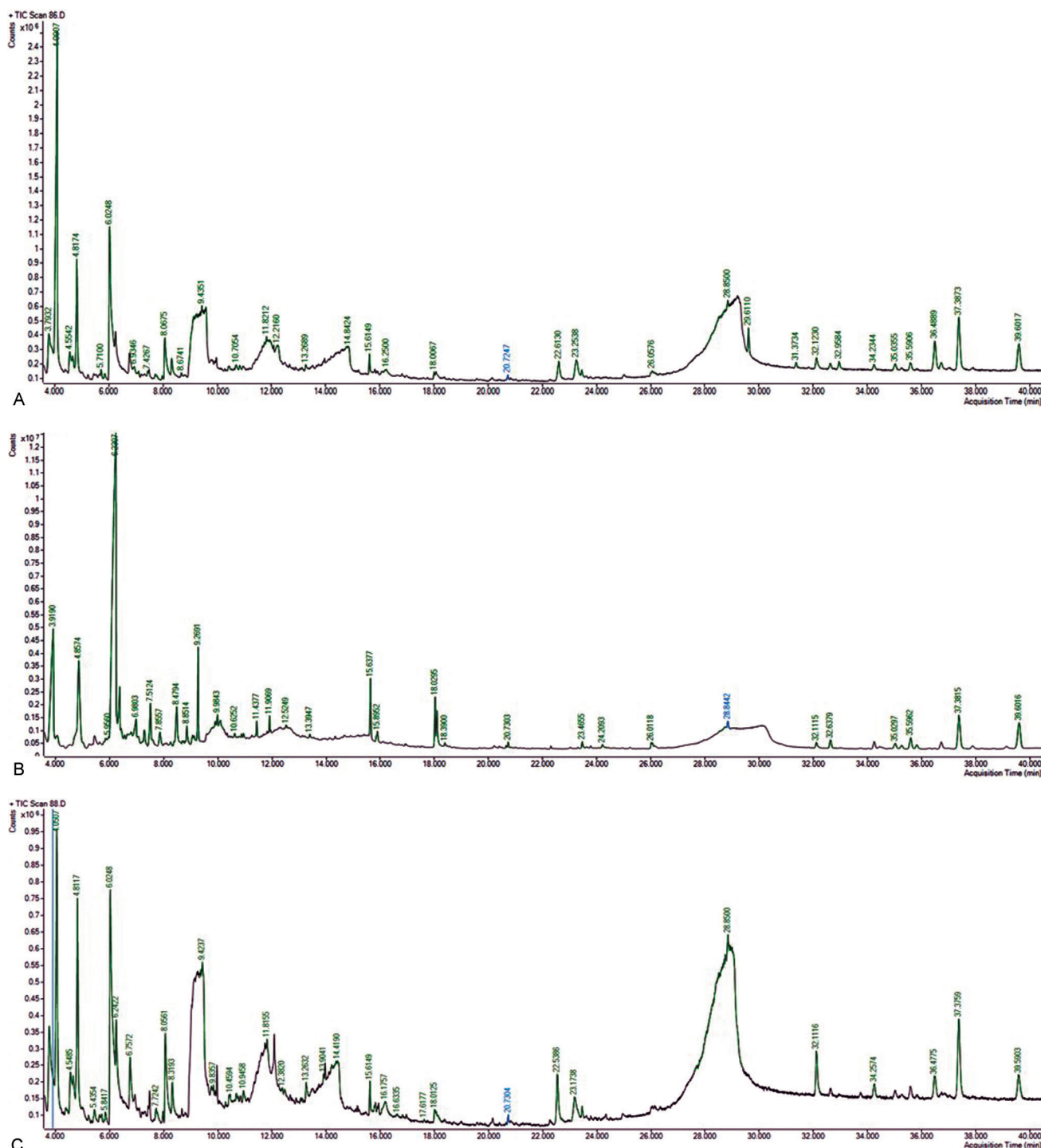


Fig. 3 GCMS/MS chromatogram (A) Co (On) 6; (B) Co (On) 5; (C) Perambalur local (Local variety).

with medicinal potentials. As a result, this native landrace must concentrate more, and the onion's shelf life can be extended. Exorbitant market prices can thus be avoided during off-seasons.

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