RESEARCH PAPER

Integrative Assessment of Phenolics, Flavonoids, and Antioxidant Capacity in Batrisu Vasanu Using Min-Max Antioxidant Composite Index

Sanket Charola[1]*; Sakshi Sahu[1]; Shraddha Vadhel[1]
[1*]Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara.
Email: sanket.charola-botany@msubaroda.ac.in

ABSTRACT

Background: A popular traditional polyherbal mixture, Batrisu vasanu lacks comprehensive antioxidant characterization despite varied marketed formulations. The present study aims to assess the phenolics, flavonoids, and flavonols alongside multi-assay antioxidant profile to rank marketed samples owing to its varied herbal composition.

Materials and methods: Using methanolic extracts, 16 Batrisu vasanu samples were evaluated for their phytochemical and antioxidant profile. Total phenolics, flavonoids, and flavonols were quantified spectrophotometrically. Furthermore, antioxidant capacity was evaluated by DPPH radical scavenging (IC_{50}), total antioxidant capacity (TAC), and Ferric iron reducing antioxidant potential (FRAP). Then to compare the antioxidant capacities of the samples, antioxidant composite index (ACI) was derived using min-max normalization across three normalized parameters namely DPPH IC_{50}, FRAP and TAC.

Results: Among the samples tested, substantial phytochemical variability was observed, with phytochemical levels ranging widely across samples. MPHS7 sample exhibited the highest phenolics, flavonoid, and flavonol contents, alongside highest FRAP and TAC values. In contrast, MPHS4 and MPHS12 showed consistently lower values of phytochemicals and antioxidant values. Pearson correlation revealed strong positive correlation between phytochemicals levels and antioxidant assay values. Using ACI rankings, MPHS7 was identified having the highest antioxidant capacity, followed by MPHS8, MPHS16 and MPHS6.

Conclusion: There was marked inter-sample variation in phytochemical levels tested, highlighting the need for quality control standardization in marketed polyherbal products. The min-max ACI model used in the study provides a robust, single-metric ranking tool for antioxidant capacity analysis.

Keywords: Antioxidant composite index (ACI), antioxidant capacity, polyherbal formulation, phenolic content, DPPH assay

How to cite this article: Charola S, Sahu S, Vadhel S. Integrative Assessment of Phenolics, Flavonoids, and Antioxidant Capacity in Batrisu Vasanu Using Min-Max Antioxidant Composite Index. Int J Drug Deliv Technol. 2026;16(29s):725-730. DOI: 10.25258/ijddt.16.29s.92.

INTRODUCTION

Despite long-standing cultural use of a popular polyherbal galactagogue Batrisu vasanu, scientific studies remain limited with respect to its antioxidant potential (Gbadamosi & Okolosi, 2013). Batrisu vasanu is widely available in market as polyherbal mixture and is used during postpartum period as traditional delicacy (Charola et al., 2021). Ethnobotanical studies describe it as restorative nutraceutical prepared from a mixture of more than thirty medicinal herbs, each contributing diverse phytochemical constituents. It is shown to benefit the growth of newborns in a cohort study (Charola et al., 2026).

Polyphenols, flavonoids, and flavonols are among the most potent class of radical scavenging and reducing plant metabolites (Jain et al., 2011). These compounds significantly contribute to the antioxidant properties of the plant extracts. It aids in preventing oxidative stress-related disorders, including cardiovascular, neurodegenerative, and metabolic diseases (Pan et al., 2011). It is important to note that conventional antioxidant assays namely Ferric reducing antioxidant potential (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and total antioxidant capacity (TAC) provide complementary insights into antioxidant properties of plant extracts (Ahmed et al., 2018; Ichikawa et al., 2003). Although it aids in categorizing antioxidant potential of the samples, it does not capture entire dimensions of antioxidant activity. It makes direct comparisons across samples a bit challenging.

The composite indices integrate normalized values from multiple assays into a single metric, help determine and compare the antioxidant potential of samples (Saleem & Jan, 2021). Min-max normalization is used to standardize heterogeneous data and generating ranking of samples by overall antioxidant strength. Marketed samples of Batrisu vasanu have shown previously that their herbal composition varies significantly, posing a risk to public health (Charola et al., 2025; Kalal & Charola, 2021). This warrants the need of an integrative approach to establish nutraceutical relevance and to evaluate antioxidant efficacy across various marketed samples. This study therefore aims to quantify phenolics, flavonoids, flavonols in marketed Batrisu vasanu samples. Further, various antioxidant assays, and min-max normalization derived composite antioxidant index will help in ranking the market-based samples.

MATERIALS AND METHODS

Sample collection

Batrisu vasanu samples were collected from crude herbs and condiment shops. The samples were collected either in raw herbs form or in powdered form both. The samples were collected on condition that they should have proper on-pack information about the ingredients. Products without any standard ingredients information on pack were excluded from the study.

Methanolic extract preparation

Dried plant material was grounded as polyherbal mixture into a fine powder. A methanolic extract was prepared with 10% w/v with powdered mixture and methanol. The mixture was kept on agitation for 24 hours on a shaker as described previously for methanolic extract preparation (Chandra et al., 2011). The extract was then passed through Whatman No. 1 filter paper to eliminate the solid fraction. Using rotary evaporator at 45 ºC and reduced pressure, the filtrate was evaporated and remaining extract was left at room temperature for 1 hour in desiccator to dry completely. The obtained dry extract was stored at 4 ºC until further use.

Estimation of total Phenol, Flavonoids and Flavonols

Using the Follin-Ciocalteu method, standard curve of Gallic acid from 10 to 100 µg was prepared (McDonald et al., 2001). Sample extracts at concentration 1 mg/ml were prepared, and total phenol was estimated at 765 nm in visible spectrophotometer. The total phenol concentration was expressed as Gallic acid equivalent (GAE) per gram of extract.

Similarly, Rutin standard curve was prepared for estimation of total flavonoids from 10 to 80 µg following the procedure described previously (Chang et al., 2020). Sample extracts were tested at 1 mg/ml concentration and reaction was read at 415 nm in visible spectrophotometer. The total flavonoids concentration was expressed as Rutin equivalents (RE) per gram of extract.

Following the protocol of flavonols estimation, 0.5 – 2.0 mg rutin standards were read at 440 nm and concentration of total flavonols were expressed as Rutin equivalents (RE) per gram of extract (Kumaran & Joel Karunakaran, 2007).

Estimation of IC_{50} value of DPPH, Total antioxidant capacity and FRAP value

DPPH % radical scavenging activity was calculated using method described earlier (Blois, 1958). Then IC_{50} value was described as concentration of sample required to scavenge 50% DPPH radical. Higher the IC_{50} value, lower is the antioxidant capacity of the sample.

Using principle of reduction capacity of an extract from Mo (VI) to Mo (V) at acidic pH, total antioxidant capacity was determined (Prieto et al., 1999). The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) per gram of extract.

Ferric ion reducing antioxidant potential (FRAP) assay was performed as capacity of extract to reduce a colorless ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to blue colored Fe^{2+}-TPTZ complex (Benzie & Strain, 1996). The FRAP value was expressed as mM Fe (II) per gram of extract.

Statistical analysis

Data was computed using spreadsheet and was analyzed using Rstudio version 2026.01.2 by Posit software. Using descriptive statistics, data was expressed as Mean ± standard deviation. Pearson correlation was performed among phenols, flavonoids, flavonols, TAC, FRAP value and DPPH IC50 values. Antioxidant composite index (ACI) was calculated from FRAP value, reversed DPPH value, and total antioxidant capacity data using min-max normalization with following formula. The normalization data ranges from 0 to 1 scale.

$$X' = \frac{X - X_{min}}{X_{max} - X_{min}}$$

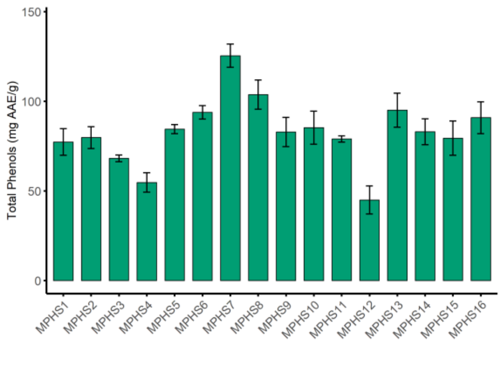
After min-max normalization, ACI was calculated with following formula.

$$ACI = \frac{FRAP' + DPPH' + TAC'}{3}$$

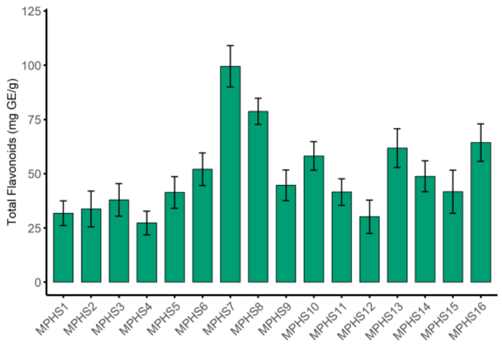
The samples with highest ACI indicates the highest antioxidant capacity in sample.

RESULTS

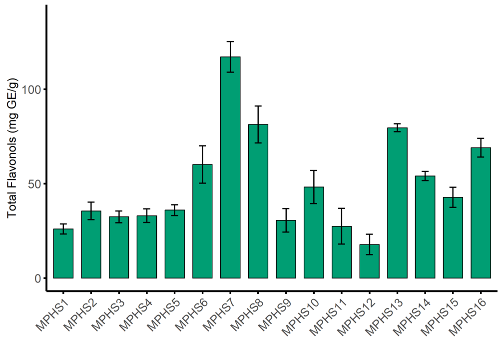
The market survey yielded total 16 different Batrisu vasanu samples, named here as marketed polyherbal samples (MPHS) and were coded as MPHS1 to MPHS16 for further analysis. Each samples were found to contain a range of 10 plants to maximum 36 plants in the polyherbal mixtures.



(A) Total phenolic content



(B) Total flavonoid content



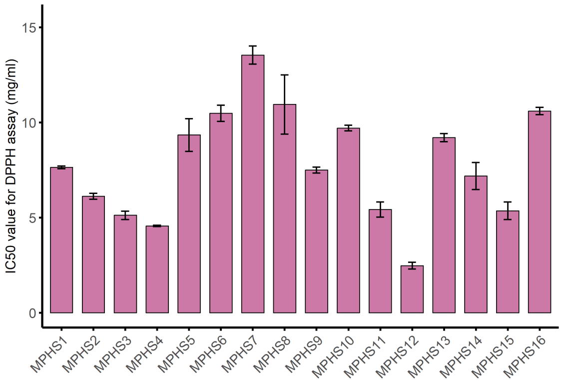
(C) Total flavonol content

Figure 1. Phytochemical profile of Batrisu vasanu samples.

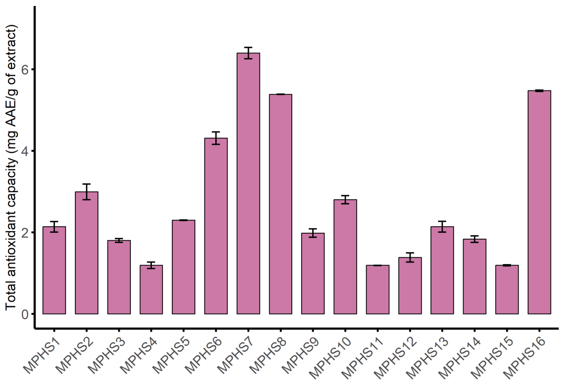
Total Phenols, flavonoids and flavonols

As shown in Figure 1(A), MPHS7, MPHS8, MPHS13 and MPHS14 were found to have highest phenol content. However, MPHS4 and MPHS12 were found to have characteristically low phenol values. Furthermore, Flavonoid content was found to be highest in MPHS7 followed by MPHS8, MPHS10, MPHS13 and MPHS16, as shown in Figure 1(B). Like phenols, total flavonols were also lowest in MPHS4 and MPHS12. Total flavonols were found to be highest in MPHS7, as indicated in Figure 1(C). However, the MPHS12 was presented with lowest flavonol content. Phytochemical analysis revealed that the total phenol content in each sample was highly varied.

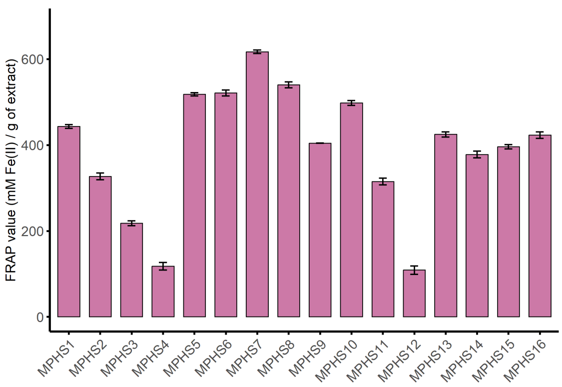
Antioxidant assays



(A) IC50 values for DPPH radical scavenging assay



(B) Total antioxidant capacity



(C) FRAP value.

Figure 2: Antioxidant assays.

Antioxidant capacity was evaluated using three different methods. As shown in Figure 2(A), IC_{50} values for MPHS12 was lowest, considered having highest antioxidant potential. However, MPHS7 was found to be the lowest in its antioxidant capacity to scavenge DPPH radical. In contrast, Total antioxidant capacity was found to be highest in MPHS7 and MPHS16, as shown in Figure 2(B). The lowest antioxidant potential was recorded for MPHS4 and MPHS11. FRAP values for MPHS4 and MPHS12 were found to be lowest (Figure 2(C)). Although MPHS7 was presented with highest FRAP value, MPHS1, MPHS5, MPHS6, MPHS8, MPHS10, MPHS13, and MPHS16 was also high, indicating its good antioxidant capacity.

Correlation of phytochemical contents with antioxidant potential

As shown in Figure 3, Total phenol content was best correlated with IC50 DPPH value, and FRAP value followed by TAC. Similarly, total flavonoids, and flavonols were best correlated with IC_{50} DPPH value, and TAC followed by FRAP value. Among all correlations, correlation between total flavonols and FRAP value was found to be lowest but was still highly correlated. The results indicated that phytochemicals were best correlated with antioxidant test parameters.

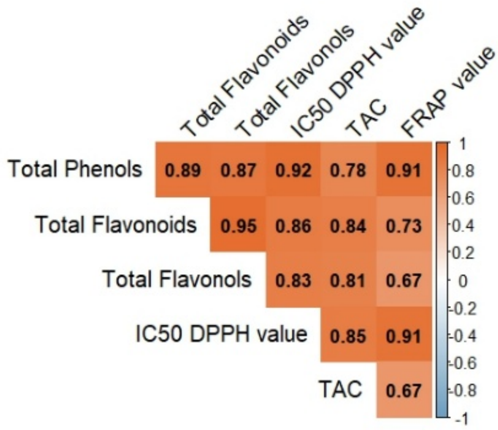


Figure 3: Pearson correlation analysis between phytochemical contents and antioxidant potential test results.

Antioxidant composite index (ACI)

Using min-max method to normalize, ACI based rank was prepared. ACI rank was calculated from IC50 of DPPH, TAC and FRAP values for all sixteen samples. It was found that MPHS7 was having highest ACI rank followed by MPHS8, MPHS16, and MPHS6. However, ACI was lowest for MPHS4 and MPHS11. The highest ACI in MPHS7 indicates that the sample is having highest antioxidant capacity among all samples tested.

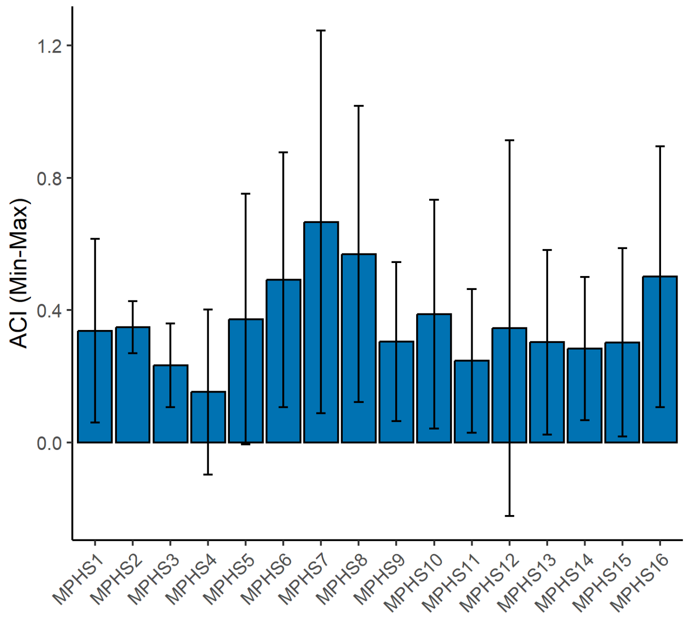


Figure 4: Calculated antioxidant composite index (ACI). Error bars indicate standard deviation of the parameter for samples.

DISCUSSION

The present study demonstrates stark variability in phytochemical contents and antioxidant parameters across collected 16 Batrisu vasanu samples. It is shown in the results that MPHS7 consistently exhibited the highest phenolic, flaovonoid, and flavonol contents. Along with phytochemicals, it also had superior FRAP and TAC values. It culminated in the highest antioxidant composite index (ACI). In contrast, MPHS4 and MPHS12 consistently showed lowest phytochemical values and antioxidant performance. Then to establish the role of phytochemicals in contributing to antioxidant performance, correlation analysis was performed. Strong correlations between total phenolics, and both FRAP and DPPH scavenging align with existing knowledge of this relationship. Due to metal chelating, and electron-donating properties, flavonoids and TAC also showed high correlation.

The study showed wider inter-sample variation in phytochemicals, indicating that it was due to differences in herbal composition, and might be due to geographic origin, post-harvest processing, or storage conditions (Sanket et al., 2025; Ștefănescu et al., 2020). Similar batch-to-batch variability in polyherbal formulations is well known from other studies as well. Here, ACI approach to integrate information from mechanistically three distinct assays (TAC, FRAP. and DPPH), provide robust, single-metric ranking tool that overcomes the limitations of any individual method. Such index would lead to regulatory and quality control frameworks for standardizing polyherbal products. The present study presented with variability in phytochemical composition of marketed Batrisu vasanu. The same was supported by varied antioxidant profile and high variability among their values, requiring an urgent attention towards the marketed polyherbal formulations. Further, the establishment of antioxidant composite index (ACI) in the present study might be a helpful tool to evaluate antioxidant capacity by indexing more than two antioxidant assay results.

REFERENCES

1. Ahmed, S., Rahman, F., Ahmed, S., & Shohael, A. (2018). Insights into the bioactive compounds, antioxidant potential and TLC profiling of different extracts of Tomato plants. Jahangirnagar University J. Biol. Sci., 7(2), 65–77. https://www.banglajol.info/index.php/JUJBS/article/view/40748
2. Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of. Analytical Biochemistry, 239(1), 70–76. https://doi.org/10.1006/ABIO.1996.0292
3. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199–1200. https://doi.org/10.1038/1811199A0
4. Chandra, K., Kumar, S., & kumar, J. (2011). Comparative standardization of a polyherbal Ayurvedic formulation Talishadi Churna. In Indian Journal of Traditional Knowledge (Vol. 10, Issue 4).
5. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis, 10(3), 3. https://doi.org/10.38212/2224-6614.2748
6. Charola, S., Adhiya, Y., Dabhi, A., & Choudhary, M. (2025). Phytochemical Profiling of the Aqueous Methanolic Extract of the Traditional Polyherbal Product Batrisu Vasanu Using GC – MS. International Journal of Innovative Science and Research Technology, 2726–2729. https://doi.org/10.38124/IJISRT/25AUG785
7. Charola, S., Tadvi, B., Albert, S., Suresh, B., & Nair, S. (2021). Analysis of a polyherbal galactagogue Batrisu vasanu, an indigenous Indian ethnomedicine. Indian Journal of Natural Products and Resources, 12(4), 610–616.
8. Charola, S., Wankhede, K., Albert, S., Nair, S., Balakrishnan, S., Patlavath, R., & Mojidra, B. (2026). Postpartum dietary supplement Batrisu vasanu improves the growth parameters of infants: A prospective cohort study. Indian Journal of Obstetrics and Gynecology Research, 13(1), 116–121. https://doi.org/10.18231/J.IJOGR.9943.1764585652
9. Gbadamosi, I., & Okolosi, O. (2013). Botanical galactogogues: nutritional values and therapeutic potentials. Journal of Applied Biosciences, 61(0), 4460. https://doi.org/10.4314/jab.v61i0.85594
10. Ichikawa, H., Wang, X., & Konishi, T. (2003). Role of component herbs in antioxidant activity of Shengmai San - A traditional Chinese medicine formula preventing cerebral oxidative damage in rat. American Journal of Chinese Medicine, 31(4), 509–521. https://doi.org/10.1142/S0192415X03001193
11. Jain, N., Ramawat, K. G., Goyal, S., & Ramawat, K. G. (2011). Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in Rajasthan. International Journal of Pharmacy and Pharmaceutical Sciences, 3(3), 248–253. https://www.researchgate.net/publication/289059015
12. Kalal, C., & Charola, S. (2021). An alarming public health concern over variability in herbal compositions of marketed immunity booster products during COVID-19: A botanical survey-based study. International Journal of Experimental Research and Review, 24, 40–50. https://doi.org/10.52756/IJERR.2021.V24.005
13. Kumaran, A., & Joel Karunakaran, R. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT, 40(2), 344–352. https://doi.org/10.1016/J.LWT.2005.09.011
14. McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 73(1), 73–84. https://doi.org/10.1016/S0308-8146(00)00288-0
15. Pan, M. H., Chiou, Y. S., Tsai, M. L., & Ho, C. T. (2011). Anti-inflammatory activity of traditional chinese medicinal herbs. Journal of Traditional and Complementary Medicine, 1(1), 8–24. https://doi.org/10.1016/S2225-4110(16)30052-9
16. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269(2), 337–341. https://doi.org/10.1006/ABIO.1999.4019
17. Saleem, S. M., & Jan, S. S. (2021). Modified Kuppuswamy socioeconomic scale updated for the year 2021. Indian Journal of Forensic and Community Medicine, 8(1), 1–3. https://doi.org/10.18231/j.ijfcm.2021.001
18. Sanket, C., Patlavath, R., Desai, M., Lency, P., Pujan, K., Afsha, A., & Charmi, P. (2025). A Comparative Qualitative Phytochemical Analysis of in-House and Commercial Polyherbal Formulations Using Thin Layer Chromatography. Indian Journal of Plant Sciences, 14, 1–5.
19. Ştefănescu, R., Tero-Vescan, A., Negroiu, A., Aurică, E., & Vari, C. E. (2020). A comprehensive review of the phytochemical, pharmacological, and toxicological properties of tribulus terrestris l. Biomolecules, 10(5). https://doi.org/10.3390/biom10050752