

Determination Of Ascorbic Acid and Total Sugar Content in Amla (*Phyllanthus Emblica*) Seed and Seed Coat Extracts by Uv-Visible Spectrophotometry

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Abstract—Objective: The present study was carried out to determine and compare the ascorbic acid (vitamin C) and total sugar content of amla (*Phyllanthus emblica*) seed and seed coat extracts using UV-Visible spectrophotometry.

Methods: Amla seed coat was extracted by the Soxhlet method and amla seed by magnetic stirrer-assisted extraction, using oxalic acid solution as the extraction solvent. Preliminary phytochemical screening was carried out on both extracts. Ascorbic acid content was estimated at 350 nm against a standard ascorbic acid calibration curve prepared in oxalic acid-EDTA solution. Total sugar content was estimated by the phenol-sulphuric acid method at 550 nm using glucose as the reference standard.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, glycosides and carbohydrates in both extracts, with a comparatively higher flavonoid and phenolic content in the seed coat. The ascorbic acid calibration curve ($y = 0.00765x + 0.3445$) gave a mean ascorbic acid content of 2.08 mg/g in the seed coat, whereas the seed extract showed absorbance values below the detection limit (BDL) of the assay. The glucose calibration curve ($y = 0.05528x + 1.1536$) gave a total sugar content of 0.319 mg glucose equivalent/g in the seed, while the seed coat extract values were below the calibration range.

Conclusion: The amla seed coat, generally discarded as a fruit-processing by-product, contains an appreciable amount of ascorbic acid, whereas the seed contains a comparatively higher amount of total sugar. UV-Visible spectrophotometry proved to be a simple, rapid and economical method for the quantitative estimation of both parameters, supporting the potential of amla seed and seed coat as low-cost natural resources for nutraceutical and pharmaceutical applications.

Index Terms—Amla, *Phyllanthus emblica*, Seed coat, Ascorbic acid, Total sugar, UV-Visible spectrophotometry, Phenol-sulphuric acid method

I. INTRODUCTION

Amla (*Phyllanthus emblica* L.), commonly known as Indian gooseberry, is an important medicinal plant of the family Phyllanthaceae, widely used in traditional Indian medicine, particularly Ayurveda. While the fruit has been extensively studied for its rich vitamin C content and nutritional value, the seed and its protective seed coat, which are generated in large quantities as by-products during fruit processing, have received comparatively little scientific attention and are usually discarded as agricultural waste.

Amla seeds are enclosed within a hard, woody seed coat and contain a range of phytochemicals, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, fatty acids, proteins and carbohydrates. These constituents are associated with antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and cytoprotective activities. The seed coat, although structurally protective in nature, has also been reported to contain polyphenolic compounds that contribute to its antioxidant and antimicrobial potential. With growing interest in the valorisation of plant-processing by-products for nutraceutical, cosmetic and pharmaceutical applications, a quantitative evaluation of the nutritional constituents of amla seed and seed coat is warranted.

Ascorbic acid (vitamin C) and total sugars are two nutritionally relevant markers that can be conveniently estimated using UV-Visible spectrophotometry, a simple, rapid, sensitive and economical analytical

technique widely employed in pharmaceutical and food analysis. The present study was therefore undertaken with the objective of estimating and comparing the ascorbic acid and total sugar content of amla seed and seed coat extracts by UV-Visible spectrophotometry, so as to evaluate their nutritional significance and their potential as value-added by-products of amla processing.

II. MATERIALS AND METHODS

Collection of plant material

Fresh amla (*Phyllanthus emblica*) fruits were collected in the month of March, based on information provided by local inhabitants. The fruits were washed thoroughly with distilled water and cut open manually, and the seeds were separated from the pulp. The seeds were washed with distilled water to remove adhering pulp and shade-dried at room temperature for 5-7 d until a constant weight was obtained. The seed coats were separated from the kernels, and both fractions were powdered separately using a mechanical grinder, passed through a 60-mesh sieve, and stored in airtight containers at room temperature until further use.

Preparation of extracts

Seed coat extract was prepared by Soxhlet extraction. Briefly, 20 g of powdered seed coat was packed in a cellulose thimble and extracted continuously with a solvent system of 0.8 g oxalic acid dissolved in distilled water and made up to 200 ml, for 6-7 h until the solvent in the siphon tube became nearly colourless. Seed extract was prepared by magnetic stirrer-assisted extraction; 20 g of powdered seed was stirred continuously with 200 ml of the oxalic acid solution using a magnetic stirrer at controlled speed and room temperature. Both extracts were filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator/water bath, made up to a final volume of 180 ml, and stored under suitable conditions until analysis.

Preliminary phytochemical screening

Both extracts were subjected to preliminary phytochemical screening for the presence of alkaloids (Dragendorff's reagent test), flavonoids (alkaline reagent test), phenols (ferric chloride test), glycosides (Keller-Killiani test) and carbohydrates (Molisch's test), using standard qualitative procedures.

Determination of λ_{max} and instrumentation

All spectrophotometric measurements were carried out on a UV-Visible spectrophotometer (GENESYS 180) using matched quartz cuvettes of 1 cm path length, over a scanning range of 200-400 nm (for ascorbic acid) and 400-600 nm (for sugar estimation), at a scan interval of 2.0 nm and medium scan speed. The instrument was blanked with the respective reagent blank prior to each set of measurements.

Estimation of ascorbic acid content

A standard stock solution of ascorbic acid (100 mg in 100 ml distilled water) was prepared, and a diluent of oxalic acid-EDTA (0.4 g oxalic acid and 0.05 g EDTA made up to 100 ml with distilled water) was used for dilutions. Standard solutions of 10, 20, 30, 40 and 50 $\mu\text{g/ml}$ were prepared by appropriate dilution of the stock with the oxalic acid-EDTA solution, and their absorbance was measured at 350 nm to construct a calibration curve. For sample estimation, 1, 2, 3, 4 and 5 ml aliquots of the seed and seed coat extracts were made up to 10 ml with the oxalic acid-EDTA solution, and the absorbance was measured at 350 nm. The concentration of ascorbic acid in the extracts was calculated using the regression equation of the standard calibration curve.

Estimation of total sugar content

Total sugar content was estimated by the phenol-sulphuric acid method of Dubois et al. A standard stock solution of glucose (10 mg in 100 ml distilled water) was used to prepare standards of 10-50 $\mu\text{g/ml}$. To 1 ml of standard or sample solution, 1 ml of 5% phenol solution was added, followed by the rapid addition of 5 ml of concentrated sulphuric acid. The mixture was allowed to stand for 10 min, shaken gently, and kept for 20-30 min at room temperature for colour development. Absorbance was measured at 550 nm against a reagent blank, and a calibration curve was constructed using the standard glucose solutions. The sugar content of the seed and seed coat extracts was calculated using the regression equation of the calibration curve and expressed as mg glucose equivalent per gram (mg GE/g) of powdered sample.

Statistical analysis

Calibration curves for ascorbic acid and glucose were constructed by linear regression analysis ($y = mx + c$) of absorbance against concentration. The resulting

regression equations were used to back-calculate the concentration of ascorbic acid and total sugar in the seed and seed coat extracts.

III. RESULTS

Preliminary phytochemical screening

Phytochemical screening of the amla seed and seed coat extracts indicated the presence of alkaloids, flavonoids, phenols, glycosides and carbohydrates in both samples. Flavonoids and phenolic compounds were present in a comparatively higher intensity in the seed coat than in the seed, while carbohydrates were present in similar intensity in both extracts (Table 1).

Table 1: Preliminary phytochemical screening of amla seed and seed coat extracts

Phytoconstituent	Test performed	Seed	Seed coat
Alkaloids	Dragendorff's reagent test	+(trace)	+(trace)
Flavonoids	Alkaline reagent test	++	+++
Phenols	Ferric chloride test	++	+++
Glycosides	Keller-Killiani test	+	++
Carbohydrates	Molisch's test	++	++

‘+’ indicates presence (trace/mild); ‘++’ moderate; ‘+++’ pronounced presence of the constituent.

Calibration curve for ascorbic acid

Standard ascorbic acid solutions of 10-50 µg/ml showed absorbance values ranging from 0.421 to 0.727 at 350 nm, increasing proportionally with concentration in accordance with the Beer-Lambert law (Table 2). Linear regression analysis gave the calibration equation $y = 0.00765x + 0.3445$, which was used for the estimation of ascorbic acid in the test extracts.

Table 2: Absorbance of standard ascorbic acid solutions at 350 nm

Standard	Concentration (µg/ml)	Absorbance
S1	10	0.421
S2	20	0.448
S3	30	0.490
S4	40	0.510
S5	50	0.727

Calibration equation: $y = 0.00765x + 0.3445$

Ascorbic acid content of amla seed coat and seed extracts

Absorbance of the seed coat extract at 350 nm increased progressively with the aliquot volume (0.435-0.614), corresponding to concentrations of 11.83-35.23 µg/ml. In contrast, the seed extract showed very low, and in most aliquots negative, absorbance values (-0.077 to 0.239), which when substituted in the calibration equation produced negative concentrations, indicating that the seed extract was below the detection limit (BDL) of the assay (Table 3).

Table 3: Ascorbic acid content of amla seed coat and seed extracts at 350 nm

Sample	Seed coat absorbance	Seed coat conc. (µg/ml)	Seed absorbance	Seed conc. (µg/ml)
1	0.435	11.83	-0.077	-55.10 (BDL)
2	0.475	17.06	0.061	-37.06 (BDL)
3	0.518	22.68	0.135	-27.39 (BDL)
4	0.566	28.95	0.194	-19.67 (BDL)
5	0.614	35.23	0.239	-13.79 (BDL)

Based on a total extract volume of 180 ml prepared from 20 g of seed coat powder, the total ascorbic acid recovered was 41.67 mg, corresponding to a mean ascorbic acid content of 2.08 mg/g of seed coat

powder. The ascorbic acid content of the seed extract could not be quantified and was reported as below detection limit (BDL) (Table 4).

Table 4: Summary of ascorbic acid content in amla seed coat and seed

Sample	Sample weight (g)	Extract volume (ml)	Total ascorbic acid (mg)	Ascorbic acid content (mg/g)
Seed coat	20	180	41.67	2.08
Seed	20	180	BDL	BDL

Calibration curve for total sugar (glucose)

Standard glucose solutions of 10-50 µg/ml showed absorbance values ranging from 1.330 to 3.633 at 550 nm by the phenol-sulphuric acid method. Linear regression analysis gave the calibration equation $y = 0.05528x + 1.1536$, used for estimating total sugar content of the extracts (Table 5).

Table 5: Absorbance of standard glucose solutions at 550 nm

Standard	Concentration (µg/ml)	Absorbance
S1	10	1.330
S2	20	2.624
S3	30	2.927

Standard	Concentration (µg/ml)	Absorbance
S4	40	3.546
S5	50	3.633

Calibration equation: $y = 0.05528x + 1.1536$

Total sugar content of amla seed coat and seed extracts

Absorbance values of the seed extract at 550 nm (2.983-3.214) corresponded to sugar concentrations of 33.09-37.27 µg/ml. In contrast, the seed coat extract showed comparatively low absorbance values (0.534-0.821), which on substitution in the calibration equation yielded negative concentrations, indicating that sugar content of the seed coat was below the calibration range of the assay (Table 6).

Table 6: Total sugar content of amla seed coat and seed extracts at 550 nm

Sample	Seed coat absorbance	Seed coat conc. (µg/ml)	Seed absorbance	Seed conc. (µg/ml)
1	0.534	-11.21 (BDL)	2.983	33.09
2	0.576	-10.45 (BDL)	3.058	34.45
3	0.630	-9.47 (BDL)	3.144	36.00
4	0.763	-7.07 (BDL)	3.160	36.29
5	0.821	-6.02 (BDL)	3.214	37.27

The mean sugar concentration of the seed extract was 35.42 µg/ml. Based on a total extract volume of 180 ml prepared from 20 g of seed powder, the total sugar recovered was 6.38 mg, corresponding to a total sugar

content of 0.319 mg glucose equivalent/g (mg GE/g) of seed. The sugar content of the seed coat extract could not be quantified and was reported as below detection limit (BDL) (Table 7).

Table 7: Summary of total sugar content in amla seed and seed coat

Sample	Sample weight (g)	Extract volume (ml)	Total sugar (mg)	Sugar content (mg GE/g)
Seed	20	180	6.38	0.319
Seed coat	20	180	BDL	BDL

Photographic observations

Fig. 1: Amla seed coat



Fig. 2: Amla seed



Fig. 3: Soxhlet extraction assembly used for extraction of amla seed coat

IV. DISCUSSION

The present study demonstrates a clear qualitative and quantitative distinction between amla seed and seed coat with respect to ascorbic acid and total sugar content. Phytochemical screening showed a comparatively higher intensity of flavonoids and phenolic compounds in the seed coat, which is consistent with earlier reports describing the seed coat as a rich source of polyphenolic antioxidants relative to the seed kernel.

The seed coat extract showed a measurable and concentration-dependent increase in absorbance at 350 nm, yielding a mean ascorbic acid content of 2.08 mg/g. Although this is considerably lower than the ascorbic acid content typically reported for amla fruit pulp, its presence in the seed coat is notable because this fraction is routinely discarded as agricultural waste during fruit processing. In contrast, the seed extract produced absorbance values close to or below the reagent blank at 350 nm, resulting in negative calculated concentrations; this was interpreted as the ascorbic acid content of the seed being below the detection limit of the present assay rather than a true absence of the analyte, and more sensitive methods (e.g., titrimetric or HPLC-based estimation) may be required to quantify it accurately.

For total sugar estimation, the trend was reversed: the seed extract showed strong, concentration-appropriate absorbance at 550 nm, giving a total sugar content of 0.319 mg GE/g, whereas the seed coat extract produced absorbance values below the linear range of the glucose calibration curve. This is consistent with the compositional expectation that the seed kernel, being the storage tissue of the embryo, is comparatively richer in carbohydrates, while the lignified, fibrous seed coat is comparatively richer in structural and phenolic constituents rather than free sugars.

Taken together, these findings indicate that the seed coat and seed of amla are compositionally

complementary by-products: the seed coat is a more promising source of natural antioxidant/ascorbic acid-related constituents, while the seed is a more suitable source of carbohydrate-related constituents. Both fractions, currently treated as processing waste, may therefore be valorised separately depending on the intended application, supporting the broader goal of sustainable utilisation of amla-processing by-products in nutraceutical and pharmaceutical formulations.

A limitation of the present study is that absorbance values falling outside the linear range of the respective calibration curves could only be reported as below detection limit (BDL) rather than as precise numerical values; use of narrower, matrix-matched calibration ranges or more sensitive analytical techniques is recommended in future studies to fully quantify these low-abundance fractions.

V. CONCLUSION

The present study successfully determined and compared the ascorbic acid and total sugar content of amla (*Phyllanthus emblica*) seed and seed coat extracts using UV-Visible spectrophotometry. The seed coat was found to contain a comparatively higher amount of ascorbic acid (2.08 mg/g), while the seed contained a comparatively higher amount of total sugar (0.319 mg GE/g). UV-Visible spectrophotometry proved to be a simple, rapid, economical and reliable method for the quantitative estimation of both parameters. The findings support the potential of amla seed and seed coat, which are ordinarily discarded as agricultural by-products, as valuable and low-cost natural resources for use in herbal formulations, nutraceuticals and functional foods. Further studies employing more sensitive and specific analytical techniques are recommended to comprehensively characterise the phytochemical profile and therapeutic potential of these under-utilised plant materials.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

[Author 1] conceived and designed the study, performed the extraction, phytochemical screening and spectrophotometric analysis, and drafted the manuscript. [Author 2/Guide] supervised the work, contributed to data interpretation, and critically reviewed the manuscript. All authors read and approved the final manuscript. (Please edit to reflect the actual contribution of each author.)

AI USE STATEMENT

An AI-assisted tool (Claude, Anthropic) was used to help reorganise and format the authors' original project report/data into the journal's structured manuscript format (IMRaD structure, abstract, tables and reference formatting) and for language editing. The authors reviewed, verified and edited all AI-assisted output against their original laboratory data and take full responsibility for the accuracy and content of the final manuscript.

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REFERENCES

- [1] Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem.* 1956;28(3):350-6.
- [2] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem.* 1959;31(3):426-8.
- [3] Sadasivam S, Manickam A. *Biochemical methods*. 3rd ed. New Delhi: New Age International Publishers; 2008.
- [4] Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 3rd ed. London: Chapman and Hall; 1998.
- [5] Beckett AH, Stenlake JB. *Practical pharmaceutical chemistry*. 4th ed. New Delhi: CBS Publishers and Distributors; 2001.

- [6] Sharma BK. Instrumental methods of chemical analysis. 24th ed. Meerut: Goel Publishing House; 2007.
- [7] Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th ed. Mumbai: Himalaya Publishing House; 2007.
- [8] Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed. Belmont: Thomson Brooks/Cole; 2007.
- [9] Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. New Delhi: CBS Publishers; 1986.
- [10] Ranganna S. Handbook of analysis and quality control for fruit and vegetable products. 2nd ed. New Delhi: Tata McGraw-Hill; 1986.
- [11] AOAC International. Official methods of analysis. 21st ed. Gaithersburg, MD: AOAC International; 2019.
- [12] Indian Pharmacopoeia Commission. Indian pharmacopoeia. Ghaziabad: IPC; 2022.
- [13] British Pharmacopoeia Commission. British pharmacopoeia. London: TSO; 2023.
- [14] United States Pharmacopeial Convention. United States pharmacopeia-national formulary (USP-NF). Rockville, MD: USP; 2023.
- [15] Trease GE, Evans WC. Trease and Evans pharmacognosy. 16th ed. London: Saunders Elsevier; 2009.
- [16] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2021.
- [17] Khandelwal KR. Practical pharmacognosy: techniques and experiments. 30th ed. Pune: Nirali Prakashan; 2019.
- [18] Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. 2nd ed. Berlin: Springer; 1996.
- [19] Jain SK. Medicinal plants. New Delhi: National Book Trust; 1996.
- [20] Anonymous. The wealth of India: raw materials. Vol. VIII. New Delhi: CSIR; 2003.
- [21] Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Dehradun: International Book Distributors; 1999.
- [22] Nadkarni KM. Indian materia medica. Vol. I. Mumbai: Popular Prakashan; 2009.
- [23] Wallis TE. Textbook of pharmacognosy. 5th ed. New Delhi: CBS Publishers; 2005.
- [24] World Health Organization. Quality control methods for herbal materials. Geneva: WHO; 2011.