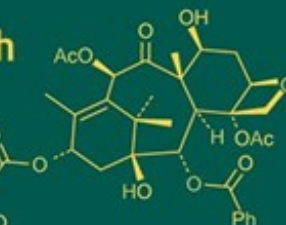


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
Indexing: Embase
IJABR 2026; SP-10(9): 2280-2283
www.biochemjournal.com
Received: 13-07-2026
Accepted: 24-08-2026
Published: 26-09-2026

Amruta P Belode

Department of Food
Chemistry and Nutrition,
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Kailash S Gadhe

Department of Food
Chemistry and Nutrition,
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Rajesh B Kshirsagar

Department of Food
Engineering, College of Food
Technology, VNMKV,
Parbhani, Maharashtra, India

Girish M Machewad

Department of Food Business
Management, College of Food
Technology, VNMKV,
Parbhani, Maharashtra, India

Formulating functional confectionery: biochemical characterization and antioxidant properties of *Terminalia arjuna*-infused milk chocolate

Amruta P Belode¹, Kailash S Gadhe², Rajesh B Kshirsagar³, Girish M Machewad⁴

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sab.10166>

ABSTRACT

Medicinal plants are of prime use in today's world. The incorporation of these medicinal plants in food applications provide us with the foods with high amount of nutrients and bio-active compounds. The *Terminalia arjuna* is most widely used medicinal tree for its benefits. The functional chocolates were prepared by using *Terminalia arjuna* bark powder by giving the alcohol treatment (70% food grade ethanol) to it. The *Terminalia arjuna* bark powder was added in chocolate at the level of 0%, 1%, 2% and 3%.

The samples were organoleptically evaluated for color and appearance, taste, flavor, texture and overall acceptability for T_0 , T_1 , T_2 and T_3 samples. The sample with 2% *Terminalia arjuna* bark powder (T_2) was highly acceptable. The proximate composition of the selected functional chocolate was found to be moisture 0.62%, fat 30.51%, protein 7.40%, fiber 1.60% and carbohydrate 5.01%. The functional analysis of the standardized chocolate showed the Total Phenolic Content 5.28 mg GAE/g and the Total Flavonoid Content 19.56%. The tannin content was found 1.31%, saponin 0.030%, alkaloid content 0.052% and the overall *anti*-oxidant activity at 24.60% by DPPH radical scavenging activity.

Keywords: *Terminalia arjuna*, Alcohol treatment, Bio-active compounds, Functional chocolate, *Anti*-oxidant activity

Introduction

Medicinal plants are the conventional source of medicine for healthcare. According to World Health Organization (WHO) 2002 report, about 80% of the global population relies on traditional medicine for some healthcare purposes and nearly 60% Indian rural population use herbal remedies as their main treatment. The interest in medicinal plants attributed to their effectiveness, no or fewer side effects, cultural acceptance, increasing healthcare costs, etc. Medicinal plants possess compounds that have interacting factors, including therapeutic potential, bioactive compounds, pharmaceutical and as a nutraceutical supplement. Several studies reported the medicinal plants potential and investigation for development of value added or functional products.

Terminalia arjuna (Roxb.) Wt. & Arn. is one such tree, belonging to the family Combretaceae. It is a deciduous tree native to the Australia, Asia and Africa (Sivalokanathan *et al.*, 2006) [1]. Whereas in India, it is present along the river banks across Maharashtra, Madhya Pradesh, West Bengal, Uttar Pradesh and Odisha. *Terminalia arjuna* has been reported to possess biological activities, antiviral, anticancer, antibacterial and antimalarial (Cock, 2015) [2], wound-healing properties (Amalraj & Gopi, 2017). The different parts of the *Terminalia arjuna* tree (viz. fruits, leaves, stem bark) exhibit distinct therapeutic profiles. phytochemical density in *Terminalia arjuna* supports for its development as a plant-derived functional ingredient. The study by Mitra *et al.* (2005) [3] reported that stem bark of *Terminalia arjuna* is rich in bioactive compounds, including triterpenoids, glycosides, flavonoids, tannins, and various minerals. Despite its immense therapeutic potential, the direct consumption of *Terminalia arjuna* bark is limited because of its bitter taste, coarse texture and poor consumer acceptability.

Chocolate is the widely consumed confectionery product across globe. It is a complex matrix of cocoa solids, sugar and milk components dispersed in continuous fat phase of cocoa butter and milk fat. Depending on its composition they are generally classified as dark, milk and white chocolate. Chocolate possess the favorable sensory properties as well as market acceptance, it offers a suitable platform for incorporating bioactive compounds, enabling the formation of nutritionally enhanced, value-added products (Singh *et al.*, 2024) [4]. The incorporation of herbal plant material like *Terminalia arjuna* is therefore an emerging approach for value-added functional product. In this research, the milk compound chocolate was used as a base ingredient and the *Terminalia arjuna* bark powder was incorporated by substituting its part.

Corresponding Author:**Amruta P Belode**

Department of Food
Chemistry and Nutrition,
College of Food
Technology, VNMKV,
Parbhani, Maharashtra,
India

Materials and Methodology

Materials

The research study was conducted using raw materials such as *Terminalia arjuna* bark was collected from campus of VNMKV, Parbhani. The required milk chocolate was procured from the local market of Parbhani. Food grade ethanol was made available in Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani.

Methodology

A) Cutting, cleaning, size reduction, and sieve analysis

1. Cutting: The stem bark of *Terminalia arjuna* was detached from the matured tree with sterile knife. The collected sample was authenticated by appropriate authority before experimental processing.

2. Cleaning: The collected bark was washed thoroughly to remove the adhering dust, soil and extraneous matter. Then was oven dried at controlled temperature.

3. Pulverization: The pulverization of the dried bark was carried out using the laboratory-scale grinder.

4. Sieving: The grounded *Terminalia arjuna* bark powder was passed through a specific mesh size to gain homogeneous, and uniform particle size.

B) Pre-treatment (alcohol treatment)

The 150 ml of 70% Ethanol (food grade) solution was prepared and placed in extraction vessel and the *Terminalia arjuna* bark powder (30 g) was added. Cold maceration was carried out at room temperature ($25 \pm 2^\circ\text{C}$) for 24 hr. with intermittent shaking. The mixture was filtered and the resulting filtrate was concentrated and dried in hot air oven at $40\text{--}45^\circ\text{C}$. The dried extract was ground into a homogeneous powder as mentioned by (Parmar *et al.*, 2017) [5].

C) Preparation of functional chocolate

Table 1. Formulation of functional chocolate

Ingredients	<i>Terminalia arjuna</i> powder (g)	Milk chocolate (g)
T ₀ (Control)	0	100
T ₁	1	99
T ₂	2	98
T ₃	3	97

Where,

T₀: 100g milk chocolate, without *Terminalia arjuna* bark powder

T₁: 99g milk chocolate + 1g *Terminalia arjuna* bark powder

T₂: 98g milk chocolate + 2g *Terminalia arjuna* bark powder

T₃: 97g milk chocolate + 3g *Terminalia arjuna* bark powder

The functional chocolate was prepared by melting milk chocolate by double boiling method and alcohol treated *Terminalia arjuna* bark powder was added as per the formulations. The variations in quantity of *Terminalia arjuna* powder was at the level of 0%, 1%, 2% and 3%. The chocolates after demoulding was packed in chocolate wrapper and stored in PET container.

Weighing of ingredients

↓

Melting of compound chocolate (double boiler)

↓

Add *Terminalia arjuna* bark powder (alcohol treated)

↓

Mixing and homogenization

↓

Moulding

↓

Cooling/setting

↓

Demoulding

↓

Packaging and storage

Flow sheet 1: Preparation of functional chocolate

Sensory evaluation of functional milk chocolate

The sensory characteristics was studied by the 10 trained panelists using the 9-point hedonic scale for prepared samples (T₀, T₁, T₂, T₃). The sensory attributes evaluated included color, appearance, taste, flavor and overall acceptability.

Evaluation of chemical composition of functional chocolates

The moisture content by hot air oven method (AOAC, 2000) [6], fat using soxhlet apparatus (AACC, 2000) [7], protein estimated by Micro-kjeldhal apparatus as described in AACC (2000) [7], ash, crude fiber AOAC (2000) [6] and carbohydrate by difference method was evaluated.

Evaluation of functional properties of functional chocolates

The Total Phenolic Content (TPC) was determined using Folin-Ciocalteu colorimetric method as described by Sharma *et al.* (2023). Total Flavonoid Content (TFC) aluminium chloride method outlined by Chang *et al.* (2002) [8]. Tannin was determined using tritrimetric protocol given by Sharma *et al.* (2023). Saponin determined gravimetrically using the protocol given by Obadoni and Ochuko (2001). For alkaloid and antioxidant activity, gravimetric method described by Harborne (1998) [9] and DPPH scavenging activity in percent using method by Brand Williams *et al.*, 1995 [10].

Statistical analysis

The results were obtained in triplicates ($n = 3$) and expressed as a mean $\pm$ standard deviation.

Result and Discussion

Organoleptic evaluation of functional milk chocolate

The organoleptic evaluation of functional chocolate sample were evaluated for control and other prepared samples. The all samples were evaluated for color and appearance, taste, flavor, texture and overall acceptability. The sample T₂ out scored all the other samples, securing the highest mean scores for color and appearance (8.8), taste (8.3), flavor (8.1), texture (8.3) and overall acceptability (8.4). The control sample (T₀) and selected sample (T₂) were further evaluated for nutritional and functional analysis.

Table 2. Organoleptic evaluation of functional chocolates

Sample Code	Color and Appearance	Taste	Flavor	Texture	Overall Acceptability
T ₀ (Control)	8.7	7.8	7.8	7.8	8.0
T ₁	8.6	8.2	7.9	8.2	8.2
T ₂	8.8	8.3	8.1	8.3	8.4
T ₃	8.3	8.0	7.3	7.8	7.9

* Each value is the mean value of all sensory reports

(continued on next page)

Proximate of prepared functional chocolate**Table 3.** Proximate composition of prepared functional chocolate

Parameter (%)	T ₀ (Control)	T ₂ (Selected)
Moisture	0.55 ± 0.06	0.62 ± 0.08
Fat	31.43 ± 0.82	30.51 ± 0.74
Protein	7.62 ± 0.36	7.40 ± 0.14
Ash	1.23 ± 0.07	1.89 ± 0.20
Fiber	1.70 ± 0.12	1.60 ± 0.17
Carbohydrate	57.47 ± 1.94	58.01 ± 2.03

* Each value is average of three determinations

The proximate composition of prepared functional chocolate was carried out to determine the changes occurred by addition of *Terminalia arjuna* bark powder in chocolate. The control sample (T₀) and selected sample (T₂) were evaluated.

The chemical composition data revealed that, the moisture content rises from 0.55% in T₀ to 0.62% in T₂ sample, as the *Terminalia arjuna* bark powder is hygroscopic in nature and it traps the atmospheric air during mixing and processing stages. The fat content was found to be at 31.43% in T₀ and 30.51% in T₂. The reason is that as the bark powder is incorporated in chocolate, a part of it chocolate was replaced. The arjuna powder as very little amount of fat, by substituting the fat rich matrix of chocolate it lowers overall fat percentage in finished chocolate. The similar trend was observed in case of protein at 7.60% in T₀ and 7.40% in T₂ and for fiber T₀ at 1.70% and 1.60% for T₂ sample. The ash content increased due to the fact that the *Terminalia arjuna* bark powder is rich in minerals like calcium and magnesium, that directly increases the ash content of the functional chocolate. The carbohydrate content increased from 57.47% in T₀ to 58.01% in T₂, due to the reason that bark of *Terminalia arjuna* has high carbohydrate fraction.

Determination of functional properties of standardized chocolates**Table 4.** Functional properties of standardized chocolates

Functional Property	T ₀ (Control)	T ₂ (Selected)
Total Phenolic Content (mg GAE/g)	2.90 ± 0.10	5.28 ± 0.13
Total Flavonoid Content (mg QE/mg)	6.37 ± 0.17	11.24 ± 0.27
Tannin (%)	0.47 ± 0.14	1.3 ± 0.20
Saponin (%)	-	0.030 ± 0.01
Alkaloid (%)	0.050 ± 0.02	0.052 ± 0.02
Anti-oxidant activity (%)	23.15 ± 1.03	24.60 ± 1.14

* Each value is average of three determinations

The functional properties of the standardized functional chocolate are tabulated in Table 4. The Total Phenolic Content of T₀ sample was found to be 2.90 mg GAE/g and T₂ sample was found to be 5.28 mg GAE/g. While the Total Flavonoid Content was 6.37 mg QE/mg for T₀ sample and 11.24 mg QE/mg for T₂ sample. The increase in the values of TPC and TFC indicated the evidence of polyphenols in *Terminalia arjuna* bark powder was migrated successfully into the functional chocolate.

The tannin content was found at 0.47% in T₀ to 1.31% in T₂. While the addition of *Terminalia arjuna* bark powder introduced measurable trace level of saponins 0.030% in the final chocolates an active cardiotonic compound that was completely absent in control samples. This targeted addition of tannins and saponins successfully drove up the overall anti-oxidant activity, which climbed to 24.60% in T₂ from 23.15% in T₀. The antioxidant activity of compound chocolate was less compared to cocoa due to the processing steps, while the cocoa

itself is rich in antioxidants. In contrast, the alkaloid content was 0.050% in T₀ and 0.052% in T₂, as the alkaloids content in *Terminalia arjuna* bark powder is negligible and the cocoa solids drive the baseline of the chocolate. These balanced functional updates line up well with established herbal food delivery frameworks published by Ghotkar *et al.* (2025) and Meena *et al.* (2020).

Mineral composition of standardized chocolates**Table 5.** Mineral composition of standardized chocolates

Mineral (mg/100g)	T ₀ (Control)	T ₂
Calcium	165.25 ± 0.84	164.73 ± 0.96
Magnesium	56.22 ± 0.49	60.28 ± 0.78
Potassium	328 ± 1.80	321.94 ± 1.65
Sodium	125.25 ± 0.53	123.14 ± 0.63
Phosphorous	233.40 ± 0.79	233.97 ± 1.35
Zinc	150.23 ± 0.81	147.13 ± 0.77
Calcium	165.25 ± 0.84	164.73 ± 0.96

The data in tabulated in Table 5 showed the mineral profile breakdown of the standardized chocolates by addition of *Terminalia arjuna* bark powder. The magnesium and phosphorous content was 56.22 and 233.40 mg/100g for T₀ and 60.28 and 233.97 mg/100g for T₂ sample, as *Terminalia arjuna* bark powder is rich in macro-minerals. The calcium, potassium and zinc was reduced to 164.73, 321.94 and 147.13 mg/100g for T₂ from 165.25, 328 and 150.23 mg/100g for T₀ sample, due to dairy ingredient like milk powder in original chocolate compound. In case of sodium it was 125.25 for T₂ and 123.14 mg/100g for T₀ sample.

Conclusion

The research is going and showed immense potential of the medicinal plants for functional food applications. The *Terminalia arjuna* is rich source of essential nutrients as well as bioactive compounds, incorporating it in food make it an excellent ingredient for enriching food with its beneficial nutrients. The functional chocolate was prepared by giving alcohol treatment to *Terminalia arjuna* bark powder. The chocolate with 2% addition of *Terminalia arjuna* bark powder showed enhancement in nutrients, mineral and functional profiles of it. The confectionery products are willingly consumed by children, teenagers and adult. Therefore, the standardized functional chocolate offers a highly viable strategy to enrich them with antioxidant, cardioprotective and nutrient-dense properties.

References

1. Sivalokanathan S, Ilayaraja M, Balasubramanian MP. Antioxidant activity of *Terminalia arjuna* bark extract on N-nitrosodiethylamine-induced hepatocellular carcinoma in rats. *Mol Cell Biochem.* 2006;281(1-2):87-93.
2. Cock IE. The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). *Inflammopharmacology.* 2015;23(5):203-29.
3. Mitra A, Nandi S. Phytochemical and mineral evaluation of *Terminalia arjuna* stem bark extracts. *Indian J Pharm Sci.* 2005;67(3):345-348.
4. Singh PK, Khedkar RD, Chandra S. Chocolate: An overview of functional potential and recent trends in fortification. *Braz J Food Technol.* 2024;27:e2023118.
5. Parmar P, Khamrui K. Development of process for the production of arjuna herbal ghee from buffalo milk. *Indian J Anim Sci.* 2017;87(2):203-207.
6. Horwitz W, editor. Official methods of analysis of AOAC International. 17th ed. Gaithersburg (MD): AOAC International; 2000.

7. American Association of Cereal Chemists. Approved methods of the American Association of Cereal Chemists. 10th ed. St. Paul (MN): AACC; 2000.
8. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-182.
9. Harborne JB. Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition. London: Chapman and Hall Ltd; 1998:21-72.
10. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Science and Technology. 1995;28:25-30.
11. Amalraj A, Gopi S. Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: A review. J Tradit Complement Med. 2016;7(1):65-78.
12. Obadoni B, Ochuko P. Phytochemical Studies And Comparative Efficacy Of The Crude Extracts Of Some Haemostatic Plants In Edo And Delta States Of Nigeria. Global Journal of Pure and Applied Sciences. 2002;8(2).
13. Sharma T, Gamit R, Acharya R, Shukla VJ. Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of *Byttneria herbacea* Roxb. AYU. 2021;42(3):143-147.

How to cite this article: Belode AP, Gadhe KS, Kshirsagar RB, Machewad GM. Formulating functional confectionery: biochemical characterization and antioxidant properties of *Terminalia arjuna*-infused milk chocolate. *International Journal of Advanced Biochemistry Research*. 2026;SP-10(9):2280-2283. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sab.10166>