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Physicochemical, antioxidant and sensory profiling of dark chocolate fortified with *Terminalia Arjuna* Bark powder

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ABSTRACT

Medicinal plants have received considerable attention around the world as the potential source of bioactive compounds. *Terminalia arjuna* is the most widely used medicinal tree for its immense potential for helping in curing various diseases. The functional chocolates prepared using *Terminalia arjuna* bark powder in dark chocolate compound. The alcohol treated *Terminalia arjuna* bark powder was added at the level of 0%, 1%, 2% and 3% by replacing chocolate compound.

The samples were analyzed for sensory characteristics and the sample with 2% *Terminalia arjuna* bark powder (S₂) was found highly acceptable. The proximate composition of the selected chocolate was moisture content of 0.41%, fat content 33.60%, protein content 3.27%, ash content 1.24%, fiber 4.42% and carbohydrate found to be 57.04%. The functional characterization showed the Total Phenolic Content of 7.68 mg GAE/g, Total flavonoid content 19.56 mg QE/mg, tannin content at 2.01%, alkaloid 0.16% and anti-oxidant activity by DPPH radical scavenging method was 52.72%.

Keywords: Medicinal plant, *Terminalia arjuna*, Bioactive Compounds, Dark Chocolate, DPPH

Introduction

Medicinal plants have long served as an important source of therapeutic agents worldwide, contributing to the growing interest in safe, effective, and economically accessible healthcare alternatives with fewer adverse effects (Jain *et al.*, 2009) [1]. According to the World Health Organization (WHO), nearly 80% of the world's population depends on traditional systems of medicine to meet their primary healthcare needs (WHO, 2002) [2]. In developing nations like India, approximately 60% of the rural population turns to herbal remedies as a primary treatment source due to rising healthcare costs and cultural acceptance (WHO, 2002; Amalraj *et al.*, 2017; Sewell *et al.*, 2014; Sharma *et al.*, 2008) [2, 3, 4, 5]. Factors such as the increasing cost of conventional medical treatment, accessibility, and longstanding cultural acceptance have contributed to the continued use of herbal therapies (WHO, 2002; Amalraj & Gopi, 2017; Sewell *et al.*, 2014; Sharma *et al.*, 2008) [4]. However, only a small fraction of these bioactive rich resources has been scientifically explored or integrated into modern healthcare systems (Jeyanthi *et al.*, 2013; Das *et al.*, 2015; Kaushik *et al.*, 2020; Jagtap *et al.*, 2009; Kumar *et al.*, 2020) [7, 6, 8, 9, 10].

Terminalia arjuna (Roxb.) Wt. & Arn. (Combretaceae), widely referred to as Arjuna, is a notable deciduous tree found in seasonal forests and semi-arid regions of Asia, Africa, and Australia (Gaikwad *et al.*, 2018; Sivalokanathan *et al.*, 2006) [11, 12]. In the Indian subcontinent, it is particularly abundant along riverbanks, where it thrives in riparian soils (Sivalokanathan *et al.*, 2006) [12]. The stem bark of *T. arjuna* is its most pharmacologically valuable part (Warrier *et al.*, 1993-1996) [13]. Biochemically, the bark boasts a high density of secondary metabolites including phenolic compounds, flavonoids, tannins, triterpenoids, glycosides, saponins, and sterols, which impart exceptional antioxidant, free radical scavenging, cardioprotective, antimicrobial, and anti-inflammatory activities (Warrier *et al.*, 1993 [13]–1996; Dhingra *et al.*, 2013 [14]; Sivalokanathan *et al.*, 2006 [12]; Cock, 2015 [15]; Jain *et al.*, 2009 [1]; Viswanatha *et al.*, 2010 [16]; Anka *et al.*, 2019 [17]; Gupta & Jain, 2011) [18].

Confectionery products, particularly chocolate, represent highly popular food matrices globally (Goldstein, 2015) [19]. Modern chocolate processing creates a complex food system where cocoa solids, sugar, and milk components are dispersed in a continuous fat phase (Shafi *et al.*, 2018; Singh *et al.*, 2024; Özbal *et al.*, 2022) [21, 22, 20]. While raw cocoa naturally contains health-promoting antioxidants, standard manufacturing processes like roasting and alkalization degrade these native compounds (Shafi *et al.*, 2018; Singh *et al.*, 2024) [21]. Consequently, reformulating chocolate by incorporating external plant-derived bioactive ingredients offers an excellent platform to restore and enhance its nutritional profile (Singh *et al.*, 2024) [22]. Recent studies validate the viability of chocolate as a functional carrier, demonstrating successful fortification using

Arthrospira platensis (Özbal *et al.*, 2022) [20] as well as functional spices like liquorice, cinnamon, cardamom, and rosemary (Reddy *et al.*, 2024) [23].

Despite the profound therapeutic properties of *Terminalia arjuna* bark, its direct consumption faces steep challenges because the raw bark powder possesses an intense bitterness, severe astringency and high moisture sensitivity, which reduce consumer acceptance (Khan *et al.*, 2023; Damale *et al.*, 2026) [25, 24]. Incorporating *Terminalia arjuna* bark extract into a compound chocolate matrix strategically addresses these multi-dimensional challenges. The lipid-continuous phase of chocolate can effectively mask the undesirable sensory attributes of the bark while protecting its moisture-sensitive bioactive compounds. This formulation represents an innovative approach to creating a value-added functional confectionery product. However, creating such a product requires a systematic evaluation of processing conditions, raw material standardization, and final product quality.

Materials and Methodology

Materials

3.1 Raw materials and reagents

Healthy, mature outer bark specimen of *Terminalia arjuna* were harvested from the VNMKV, Parbhani university campus and authenticated by competent botanical authorities to ensure specimen integrity before experimental processing. Commercial-grade dark compound chocolates were procured from local commercial markets. All chemicals, solvents (including food-grade 70% ethanol and citric acid) and reagents utilized during the investigation were of analytical grade.

3.2 Equipment and instrumentation

Processing and analytical instrumentation included a laboratory-scale mixer grinder, cabinet dryer, mechanical sieve shaker, hot-air oven, digital analytical balance, and a water bath. Product characterisation was performed using a HunterLab ColorFlex EZ Colorimeter.

Processing of raw material

A. Cleaning, size reduction, and sieve analysis

The collected raw *Terminalia arjuna* bark samples were processed using the following sequence:

- 1. Cutting:** The *Terminalia arjuna* stem bark was detached from the tree using a knife.
- 2. Cleaning:** Bark samples were washed thoroughly with distilled water to remove adhering dust, soil, microbial surface contamination, and extraneous matter. Cleaned barks were oven-dried at a controlled temperature to a constant weight.
- 3. Pulverization:** The dried bark samples were subjected to mechanical size reduction using a laboratory-scale grinder.
- 4. Sieving:** Sieve analysis was conducted using a standard mechanical sieve shaker to isolate a homogenous, uniform particle size distribution and eliminate oversized fibrous particles.

B. Experimental pre-treatments and optimization

To optimize the yield and maximize the retention of bioactive compounds, the processed bark powder was evaluated under two distinct partial hydrolysis extraction pre-treatments:

Method A: Citric acid hydrolysis

3.0 g of citric acid was dissolved in 300 ml of distilled water to prepare acid solution. A 30.0 g aliquot of the processed bark powder was mixed with the prepared solution (1:10 w/v solid-to-solvent ratio) in a glass vessel. The vessel was tightly sealed and thermally incubated in a water bath at 50-60 °C for 30-45 minutes with periodic shaking. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper. The resulting filtrate was dried in an oven at 50 °C for 12-24 hours to obtain the final concentrated extract powder, as given by Puspitasari *et al.* (2025) [26] with some modifications.

Method B: Alcohol treatment (partial hydrolysis)

A 30.0 g aliquot of the fine bark powder was placed into an extraction vessel, and 150 ml of food-grade 70% ethanol was added to achieve a 1:5 w/v solid-to-solvent ratio. Extraction was carried out via maceration at room temperature (25 ± 2 °C) for 24 hours under intermittent shaking to enhance mass transfer. The mixture was then filtered through Whatman No. 1 filter paper to remove insoluble plant debris. The resulting filtrate was concentrated via solvent evaporation and dried in a hot-air oven at 40-45 °C. Finally, the dried extract was ground into a homogeneous powder and stored for further analysis (Parmar *et al.*, 2017) [27].

C. Pre-treatment selection criteria

Weight of recovered pre-treated powder

Following the execution of both pre-treatments, fractions were dried, recovered, and quantitatively evaluated for total yield percentage using the standard formula:

Weight of sample used

Yield (%) = $\times 100$

Based on comparative statistical analysis, Method B (Alcohol treatment) demonstrated a significantly superior yield profile ($P < 0.05$) compared to Method A. Consequently, the alcohol-treated substrate was selected as the optimized substrate for subsequent chocolate matrix incorporation.

D.

Recipe formulation and standardization

The functional chocolate formulations were standardized by replacing portions of the dark compound chocolates with varying concentrations (1%, 2%, and 3%) of the optimized *Terminalia arjuna* bark extract. Unfortified compound chocolates served as control.

Where,

S₀: 100g dark chocolate, without *Terminalia arjuna* bark powder

Table 1. Formulation matrix for functional milk chocolates (per 100g batch)

Ingredient Type	<i>Terminalia arjuna</i> powder (g)	Dark chocolate (g)
S ₀ (Control)	0	100
S ₁	1	99
S ₂	2	98
S ₃	3	97

S₁: 99g dark chocolate + 1g *Terminalia arjuna* bark powder

S₂: 98g dark chocolate + 2g *Terminalia arjuna* bark powder

S₃: 97g dark chocolate + 3g *Terminalia arjuna* bark powder

Preparation of functional chocolate

The functional chocolates were prepared by melting dark chocolate using double-boiler method (45-50 °C, 5-10 min). Then the alcohol treated *Terminalia arjuna* bark powder was added as per the formula-

tions. The mixture was then homogenized for 1 min and moulded into the moulds and allowed for setting of chocolates. Packed in chocolate wrapper after demoulding and stored in PET container.

The following flowchart shows the standard processing steps for preparation of functional chocolates.

Weighing of Ingredients

↓

Melting of Compound Chocolate
(Double boiler: 45-50 °C, 5-10 min)

↓
Incorporation of Alcohol-Treated Bark Powder

↓
Mixing and Homogenization (1 min)

↓
Moulding

↓
Cooling/Setting (4-10 °C)

↓
Demoulding

↓
Packaging and Storage (4 °C)

Flow sheet 1: Preparation of functional chocolate



Figure 1: Prepared functional chocolates

Sensory evaluation of functional dark chocolate

The sensorial characteristics was studied using the 9-point hedonic scale by the 10 trained panelist member for prepared samples (S₀, S₁, S₂, S₃). The sensory attributes evaluated included color and appearance, taste, flavor and overall acceptability.

Evaluation of chemical composition of functional chocolates

The moisture content was estimated by hot air oven method (AOAC, 2000) [28], fat content using Soxhlet apparatus (AACC, 2000) [29], protein content estimated by Micro-kjeldhal apparatus as described in AACC (2000) [29], ash, crude fiber AOAC (2000) [28] and carbohydrate by difference method were evaluated.

Evaluation of functional properties of functional chocolates

The phytochemical profile and antioxidant activity of the chocolates (S₀ and S₂) were evaluated using established quantitative methods. Total Phenolic Content (TPC) was determined by the Folin-Ciocalteu colorimetric assay following the protocol described by Sharma *et al.* (2023) [30], while Total Flavonoid Content (TFC) was measured using the aluminum chloride method proposed by Chang *et al.* (2002) [31]. Tannin content was quantified by the titrimetric procedure established by Sharma *et al.* (2023) [30]. Saponin and alkaloid contents were both determined gravimetrically, adhering to the methodologies of Obadoni *et al.* (2001) [32] and Harborne (1998) [33], respectively. Finally, the antioxidant activity was assessed by measuring the percent DPPH radical scavenging activity according to the method developed by Brand-Williams *et al.* (1995) [34].

Statistical analysis

Each analysis was performed three times ($n = 3$), with the results reported as mean values along with their standard deviations.

Result and Discussion

Organoleptic evaluation of functional milk chocolate

Table 2. Organoleptic evaluation of functional chocolates

Sample Code	Color and Appearance	Taste	Flavor	Texture	Overall Acceptability
S ₀ (Control)	8.4	7.9	7.9	7.8	7.9
S ₁	8.3	8.2	8.1	8.2	8.3
S ₂	8.8	8.4	8.4	8.4	8.6
S ₃	8.2	8.1	7.8	7.7	7.8

* Each value is the mean value of all sensory reports

The organoleptic sensorial analysis of the prepared chocolates was conducted using 9-point hedonic scale. The S₂ (Selected) chocolate sample outsourced all the other formulations, for color and appearance

(8.8), taste (8.4), flavor (8.4), texture (8.4) and overall acceptability (8.6).

Proximate of prepared functional chocolate

Table 3. Proximate composition of prepared functional chocolate

Parameter (%)	S ₀ (Control)	S ₂ (Selected)
Moisture	0.36 ± 0.04	0.41 ± 0.05
Fat	34.28 ± 0.93	33.60 ± 0.91
Protein	3.31 ± 0.14	3.27 ± 0.07
Ash	1.11 ± 0.15	1.24 ± 0.08
Fiber	4.51 ± 0.20	4.42 ± 0.36
Carbohydrate	56.43 ± 0.82	57.04 ± 0.83

* Each value is the mean value of all sensory reports

(continued on next page)

The proximate composition of the prepared S₀ (Control) and S₂ (Selected) chocolates was carried out to understand how *Terminalia arjuna* bark powder affects the basic nutritional setup of the final product. The prominent increase was seen in the ash content rising from 1.11 to 1.24%, mainly due to the mineral content present in the bark powder. The same trend was observed for the carbohydrate con-

tent. The fat content dropped from 34.28 to 33.60%, this was due to the amount of fat in the *Terminalia arjuna* in powder is less and substituting the chocolate with bark powder dilutes the overall fat content. Similar occurs in case of the fiber and protein content.

Determination of functional properties of standardized chocolates

Table 4. Functional properties of standardized chocolates

Functional Property	S ₀ (Control)	S ₂ (Selected)
Total Phenolic Content (mg GAE/g)	5.35 ± 0.19	7.68 ± 0.20
Total Flavonoid Content (mg QE/mg)	14.86 ± 0.51	19.56 ± 0.60
Tannin (%)	1.18 ± 0.07	2.01 ± 0.13
Saponin (%)	-	0.034 ± 0.02
Alkaloid (%)	0.17 ± 0.02	0.16 ± 0.04
Anti-oxidant activity (%)	51.30 ± 1.32	52.72 ± 1.99

* Each value is the mean value of all sensory reports

The quantitative phytochemicals of the prepared chocolates were evaluated for the presence of bioactive components in the chocolates. The phytochemical assay showed the significant and uniform increase in the active phytochemicals in the selected (S₂) chocolates. The Total Phenolic Content was 5.35 mg GAE/g increased to 7.68 mg GAE/g and Total Flavonoid Content was 14.86 mg QE/mg to 19.56 mg QE/g. This showed the evidence that the polyphenols present in *Terminalia arjuna* bark powder successfully migrated into the chocolates matri-

ces. The tannin content was increased to 2.01% from 1.81% and saponins was added by incorporating *Terminalia arjuna* bark powder, as the compound chocolates didn't have saponins in it. This addition of tannins and saponins drove up the antioxidant activity from 51.30% to 52.72%. Whereas, the alkaloid content remained almost identical in both the chocolates.

Figure 2. Functional properties of Standardized Chocolates

Mineral composition of standardized chocolates

Table 5. Mineral composition of standardized chocolates

Mineral (mg/100g)	S ₀ (Control)	S ₂
Calcium	230.26 ± 1.74	228.43 ± 1.69
Magnesium	128.41 ± 0.67	130.84 ± 0.93
Potassium	434.57 ± 2.47	425.82 ± 2.08
Sodium	22.00 ± 0.16	22.20 ± 0.24
Phosphorous	207.43 ± 1.32	208.49 ± 1.74
Zinc	170.28 ± 1.70	166.73 ± 0.95

* Each value is average of three determinations

The data highlights the upward trend for magnesium (130.84 mg/100g), sodium (22.20 mg/100g) and phosphorous content (208.49 mg/100g) in selected (S₂) variants, due to the reason that the raw *Terminalia arjuna* bark powder is naturally a rich source of these macro-minerals. While the calcium, potassium and zinc showed mi-

nor decrease into the fortified chocolates, as the small portion of cocoa solids was swapped for the herbal bark powder of *Terminalia arjuna* which slightly lowers the percentage of these native minerals.

Figure 3. Mineral Composition of Standardized Chocolates

Color characteristics of standardized chocolates

Table 6. Mineral composition of standardized chocolates

Color value	S ₀ (Control)	S ₂
L*	22.46	19.66
a*	2.25	1.94
b*	2.74	2.40

* Each value is average of three determinations

The colorimetric values demonstrated a distinct shift towards a darker visual profile upon the addition of the *Terminalia arjuna* bark powder as L* value decreases. The a* value decreases meaning that the red shifted slightly towards the green. The b* value also decreases meaning the color value shifted from yellow to blue.

Conclusion

Terminalia arjuna is the medicinal tree that possess a wide range of biological activities like anti-oxidant, anti-viral, cardio-tonic, etc. Incorporating this in the suitable food matrix creates a food with functional benefits. The functional chocolate prepared by using *Terminalia arjuna* bark powder in dark compound chocolate enhances the overall nutritional, mineral and functional profile of the chocolate. Also the

chocolate is the confectionery preferred by children, adult and teenagers for consumption. Therefore, the standardized chocolate offers a way of enriching the chocolate with a number of beneficial properties.

Figures



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