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Characterizing Nutritional, Antioxidant and Antimicrobial Values of *Diploknema butyracea* (Roxburgh) H. J. Lam from the Chepang Community, Makwanpur, Nepal

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Abstract

The multipurpose tree *Diploknema butyracea* (Roxb. H.J. Lam), known locally as chiuri, is vital for food security and beekeeping in rural Nepal. This study examines its nutritional and phytochemical traits sourced from a Chepang community in Makwanpur, Nepal. This research focuses on macronutrients like carbohydrates, protein, fat, and ash alongside phytochemicals such as phenolic content, vitamin C, β-carotene, and lycopene. The study aimed to estimate this fruit's antimicrobial and antioxidant characteristics. The pulp and seed samples were analyzed for their nutritional and phytochemical components using standard methods (AOAC 1995). We determined the antioxidant and antimicrobial activity using the DPPH assay and agar diffusion method respectively.

This fruit has a high-fat content: 30.29% in the seed and 20.23% in the pulp. The pulp and seed also contain noteworthy levels of the total phenolic content (486.08 ± 0.006 and 182. 26 ± 0.001 mg Gallic Acid Equivalent (GAEs) /100 g), vitamin C (20.70 ± 0.002 and 19.08 ± 0.005 mg Ascorbic Acid (AA)/100 g) with trace extents of compounds lycopene, β-carotene and carotenoids. We observed the antioxidant activity at 2207 ± 0.01 g/mL in pulp and 1841.05 ± 0.77 g/mL in seed, which is a substantial value. Both were discovered to be effective against *Candida albicans* at doses ranging from 25 to 100 mg/mL. By performing this study, we concluded that *D. butyracea* is a significant food source that can also be used medically.

Keywords: antimicrobial potential, antioxidants, *Diploknema butyracea*, Nepal, nutrients, phytochemicals

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Introduction

The Chiuri tree, also known as the Indian butter tree (*Diploknema butyracea* (Roxburgh) H. J. Lam), is a medium-sized tree that thrives in the sub-Himalayan regions, specifically on open slopes at altitudes ranging from 200-1700 meters [1]. This tree is indigenous to the Himalayan belt of Asia, which encompasses countries like Nepal, India, and Bhutan [2], and grows to around 25 meters [3]. The Chiuri tree is known for its medicinal properties, including its effectiveness against rheumatism, asthma, and ulcers. In addition to its medicinal uses, the tree also serves other purposes such as cooking and lighting in Nepal [4]; the chiuri tree is considered a symbol of prosperity for many communities, particularly among the Chepang community in Nepal [5]. The *D. butyracea* species is highly valued for its economic contributions in the form of fodder, fuel wood, timber, and other products. It also has significant medicinal applications in treating conditions like itching, bleeding, and tonsil inflammation, among others. However, despite its high economic and medicinal importance, *D. butyracea* faces the threat of extinction due to human-induced pressures [6, 7].

Chiuri has a variety of ethnobotanical uses within the Chepang community, making it a significant part of their culture. The seeds of this tree are extracted to produce "Chiuri butter" or "Phulwara butter", while the fruit is consumed fresh and also distilled for alcohol, and the tree and oil-cakes serve as firewood and manure, respectively [8, 9]. In addition to its direct uses, the tree offers food and habitat for a variety of wildlife species, including bats, which are culturally significant. The community adheres to strict tree ownership rules and a cultural prohibition on tree cutting and branch lopping, contributing to the conservation of chiuri. However,

compliance with these rules is on the decline [3,10]. These ethnobotanical uses underscore the importance of *D. butyracea* in the Chepang community, serving not only as a source of nutrition and medicine but also as a vital part of their cultural practices and ecosystem.

Chiuri fruits are tender and juicy and ripen between June and August [1, 11]. Production of the fruits varies from 5 kg to 694 kg, with an average of 67.94 kg per tree [12]. These fruits and their seeds are rich in nutrients and have high levels of phytochemicals, which have antioxidant properties [13]. The fruits are consumed as food supplements while the fats from the seeds are used for cooking, lighting lamps, and making products like soap, candles, and hair oils [14] due to their substantial phytochemical and oil content [3].

Since ancient times, numerous tribal communities, such as Chepang in Nepal, have utilized *D. butyracea* for a variety of medicinal applications. This plant exhibits a range of pharmacological effects including antioxidant activity [15] from fruit pulp, antifungal activity [16] from seed extract, anti-inflammatory activity [10], and antibacterial activity [17] from stem bark extract, the seed extract also has feeding deterrent and insect growth inhibitory effects.

These wild edible fruits help meet the local population's nutritional needs while also helping to generate money through the sale of the products [18]. This tree contains a variety of nutritional components including flavonoids, tannins, glycosides, terpenoids, and carbohydrates [10]. In the Makwanpur region of Nepal, *Bauhinia variegata* L. (Orchid tree), *Dendrocalamus hamiltonii* Nees & Arn. ex Munro (Bamboo), *Diplazium esculentum* (Retz.) Sw. (Vegetable fern), *Dryopteris cochleata* (Ham. ex D. Don) C. Chr., and *Tectaria gemmifera* (Fée) Alston are some of the common wild edibles [19]. Among these, *D. butyracea*, holds a significant place due to its rich nutritional profile and various uses. This highlights the importance of wild edibles in contributing to the local diet and health.The reports regarding the nutrient, phytochemical composition, and antimicrobial properties of *D. butyracea* fruits and seeds are inadequate, and the information from this study might promote the fruit's utilization and exploration on a local and global scale. Our study's objectives were to assess pulps' and seeds' compositions, determine their antioxidant activity, and demonstrate antimicrobial activities.

Methods

This study assessed the nutritional and phytochemical content and the antimicrobial activity of *D. butyracea*. The plant extract was tested for its antimicrobial properties, and the nutrients and phytochemicals were analyzed using the Association of Official Analytical Chemists (AOAC) methods.

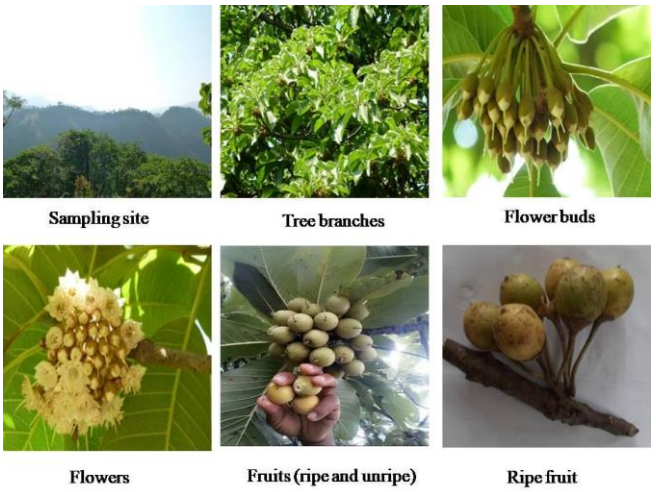


Figure 1. Sampling site and different stages of *D. butyracea* fruits

Plant material and sampling

D. butyracea fruits were harvested in July 2020 from the Silinge Village in the Raksirang Rural Municipality (27.59°N 84.86°E) of the Makwanpur district when they were ripe (**Figure 1**). The trees were recognized by examining the bark, leaves, flowers, fruits, and seeds [12]. The fruit samples were from one harvest and were mature, healthy, and had fleshy exocarp and mesocarp. These samples were then delivered to the laboratory of the Biological Resources Unit at epal Academy of Science and Technology (NAST), Nepal. They were securely packaged in zip lock bags and reached the laboratory within two days. As soon as they were received, the specimens were washed using sterile water and left to air-dry in a shaded area for two days. This was succeeded by a drying process in an oven for three days at a temperature of 40 °C. During this entire procedure, the specimens were precisely inspected for any signs of bacterial or fungal infection. The kernels were then methodically separated from the mesocarp, and both specimens were comminuted into fine particulate matter using a mortar and pestle. These granulated samples were preserved in sealed containers at a temperature of 4°C for subsequent examination.

Nutritional composition

Protein content

The protein concentration was ascertained using an adapted form of the Bradford assay [20]. To begin, a 200 mg sample of dehydrated powder was combined with 20 mL of ultrapure water (18.2 MΩ, Millipore, Milli-Q) and subjected to a 24-hour incubation at 50 °C and 100 RPM within a shaking oven (Innovative Life Science Tools, U.S.A.). After this incubation phase, the mixture was

filtered using Whatman no. 1 filter paper, and the resulting filtrate was used for protein content estimation. For the protein assessment, the filtrate was mixed with the Bradford reagent in a microfuge tube, utilizing a ratio of 1:10. This procedure was conducted in triplicate. Subsequently, each tube was thoroughly vortexed and allowed to incubate for a minimum of 5 minutes at ambient temperature. The optical density of the 200 µL aliquot from each tube was gauged at 595 nm against a blank. This was done using nanodrop (ThermoFisher Scientific, USA). A calibration curve was established using Bovine Serum Albumin (BSA) standards spanning from 0 to 400 µg/mL ($y = 0.002x + 0.143$; $R^2 = 0.99$). The derived data were subsequently intonated as g/100 g of the sample.

Carbohydrate content

The determination of carbohydrate content was executed using a colorimetric procedure that incorporated an adapted anthrone reagent, as described by Osborne and Voogt [21]. A sample weighing 0.5 g was pretreated with 15 mL of 52% (v/v) perchloric acid ($HClO_4$) and 10 mL of deionized water, then left in darkness for an 18-hour. Following this incubation, the samples were subjected to filtration, and the resulting filtrate volume was diluted by a factor of ten. A reactive solution was then prepared by combining 1 mL of the sample extract with 5 mL of a 0.1% (w/v) anthrone solution in 70% (v/v) H_2SO_4. This solution was subsequently heated in a water bath for 12 minutes, which facilitated the reaction of anthrone with the sugars, resulting in a green coloration. After the solution was cooled, a 200 µL aliquot was transferred to 96-well plates, and the optical density was observed at 630 nm using a spectrophotometer. The results were then articulated in g/100 g of the sample, based on a glucose calibration curve that spanned from 0 to 100 µg/mL ($y = 0.003x + 0.040$; $R^2 = 0.983$).

Fat content

Five-gram dehydrated specimen was transferred on glazed paper and then moved to an extraction thimble. The extraction thimble was carefully put over a layer of cotton wool in a Soxhlet extractor (S.M. Scientific Instruments, India), ensuring that the upper part of the thimble extended above the siphon. Furthermore, a dried-out round-bottomed flask was also weighed and affixed to the extractor. 100 mL of petroleum ether was added into the extractor until it commenced siphoning off, following the methodology outlined by Xiao et al. [22]. The extractor was coupled to the condenser, and the entire apparatus was set up with the flask temperature maintained within the range of 40°C to 60°C. The condenser was started, facilitating the circulation of cool water, and the extraction process was allowed to proceed for a duration of 6 hours. The ether was then evaporated using a rotary evaporator at 40 °C. In a hot air oven, the receiving flask was subjected to an hour of drying period, maintained at a 100°C temperature. After cooling, the flask was weighed again. The fat content was stated as g/100 g of the sample, as per the technique defined by Chew et al. [23].

Phytochemical analyses

Preparation of plant extracts

For the preparation of plant extracts, we used a slightly altered technique from a previously established procedure [24]. First, 1 g of dehydrated and pulverized *D. butyracea* seed and pulp were weighed individually, following the subsequent addition of methanol (20 mL). In a shaking incubator, the following mixtures were agitated at 100 RPM for 24 hours at 37 °C. Following that, the mixes were filtered through Whatman no. 1 filter paper, and the filtrates were stored at 4 °C. The resultant mixture was dried at 40°C using a rotary evaporator.

Total phenolic content (TPC)

A revised Folin-Ciocalteu test [25] was used to measure the total phenolic content. To each of the 50 µL of plant extracts, 150 µL of Folin and Ciocalteu's phenol reagent were added.

Following a 30-minute interval, a saturated sodium carbonate solution (150 µL) was added to the mixture, and the total volume was adjusted to 1500 µL with de-ionized water. After allowing the reaction to run in the dark for 90 minutes, 200 µL of the reaction mixture was transferred to a 96-well plate. The intensity of the absorption was subsequently evaluated at 725 nm. A calibration curve was constructed using gallic acid standards that ranged from 25 to 500 µg/mL ($y = 0.001x + 0.052$; $R^2 = 0.99$). The aggregates were quantified and articulated as milligrams of gallic acid equivalents (GAEs) per 100 grams of the extract.

Vitamin C content

The quantification of vitamin C was determined using a revised method derived from Klein and Perry [26]. A dry methanolic extract of *D. butyracea* weighing 50 mg was subjected to extraction in 1% meta-phosphoric acid (5 µL) for a duration of 45 minutes at ambient temperature.

The mixture was then thoroughly mixed with 900 µL of 2,6-dichlorophenolindophenol (DCPIP), adding 100 µL to each sample after the solution had been filtered through the Whatman No. 4 filter paper. The absorbance of each reactant's 200 µL portion was measured against a blank within 30 minutes at a wavelength of 515 nm in a

spectrophotometer. Each test was carried out three times. Within the 25–100 µg/mL range, a standard curve was created using ascorbic acid ($y = -0.002x + 0.692$; $R^2 = 0.981$). The result was then represented as ascorbic acid milligrams per 100 grams of extract.

β-Carotene and Lycopene contents

The quantification of β-carotene and lycopene content in the sample was conducted following a method initially developed by Nagata and Yamashita in 1992 [27]. A 100 mg dried-out methanolic extract was filtered through Whatman no. 4 filter paper after being treated for one minute with an acetone-hexane combination (4:6). Next, each sample's absorbance was measured in 200 µl portions at wavelengths of 453 nm, 505 nm, and 663 nm, respectively. These samples were all subjected to triplicate assays, and the following formulas were used to determine the amounts of β-carotene and lycopene:

For lycopene (mg/100 mL): lycopene = –0.0458 A663 + 0.372 A505 –0.0806 A453

For β-carotene (mg/100 mL): β-carotene = 0.216 A663 – 0.304 A505 + 0.452 A453

The results were subsequently displayed as micrograms (µg) of carotenoid in the sample per gram (g).

DPPH radical scavenging activity (DPPH assay)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging activity was measured using a technique that was modified from Blois [28]. A 900 µL methanolic solution containing DPPH radicals (6×10^{-5} mol/L) was mixed with 100 µL of *D. butyracea* extract at several doses. After agitating the mixture and allowing it to incubate in the dark for an hour, steady absorption levels were achieved. The depletion of DPPH radical was quantified by measuring the absorbance at 517 nm using a spectrophotometer, and the Radical Scavenging Activity (RSA) was calculated as a percentage of DPPH discoloration using a specific formula:

$$\% \text{ RSA} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

In this context, A_S represents the absorbance of the solution after the addition of a specific quantity of the sample extract, while A_{DPPH} denotes the absorbance of the DPPH solution. The extract that results in 50% of RSA, also known as the half-maximal effective concentration (EC_{50}), was determined using the RSA percentage graph plotted against the concentration of the extract. Ascorbic acid was used as the benchmark for comparison.

Screening and evaluation of antimicrobial activity of plant extracts

Preparation of stock/working solution

The crude extracts were dissolved in Dimethyl sulfoxide (DMSO) to create stock solutions with a 100 mg/mL concentration. To yield solutions with concentrations of 25 mg/mL, 50 mg/mL, and, 75 mg/mL, these stock solutions were further diluted.

Collection of standard cultures

For this study, distinct bacterial strains were chosen, including *Escherichia coli* American Type Culture Collection (ATCC 25922), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 25923) *Salmonella Typhi* (clinical isolates), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 9027), *Alkaligens faecalis* (ATCC 8750) , and one fungal strain: *Candida albicans* (ATCC 10231). After obtaining the cultures, the test bacteria were streaked on agar plates containing Nutrient Agar, followed by incubation for 24 hours at 37°C. Subsequently, the test fungi were streaked on Potato Dextrose Agar (PDA) plates and incubated at 28°C for one week. Gram staining was then performed on the isolated colonies.

Preparation of standard culture inoculums

An isolate bacterial colony designated for testing was transferred into a tube having 5 mL of sterile nutrient broth. Subsequently, it was left to incubate for a duration of 4 hours at 37°C. In the case of fungi, they were introduced into Potato Dextrose Broth (PDB) and subjected to overnight incubation at 28°C. We selected these specific conditions to align the optical density with the 0.5 McFarland standards. This alignment resulted in a final inoculum concentration of 1.5 x 10^8 Colony-Forming Units (CFU) per milliliter.

Preparation of media

The media utilized in this study were prepared per the manufacturer's procedures and recommendations by HiMedia Laboratories Pvt Ltd, India.

Antimicrobial activity assessment utilizing agar well diffusion method

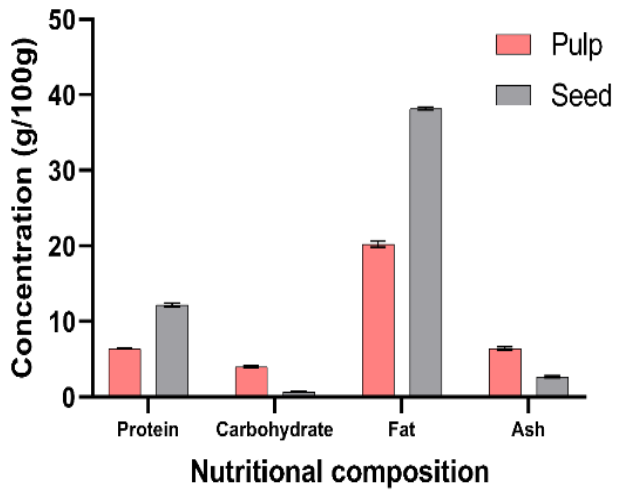
Sterile Mueller Hinton Agar (MHA) plates for bacteria and PDA plates for fungi of approximately 4 mm thicknesses were prepared. The plates were lawn cultured with a standardized microbial culture broth using sterile cotton swabs. The inoculated samples were allowed to air dry for a few minutes at room temperature (25°C), with the lids kept securely closed to maintain sterility. After drying, five 6 mm wells were created in the inoculated media using a 6 mm diameter cork borer. Each well was then filled with 50 µL of various concentrations from the plant extracts, the negative control (DMSO), and the positive control (chloramphenicol 30 mcg for bacteria and itraconazole 10 mcg for fungal isolates). The plates

were left to diffuse for ~30 minutes at a room temperature of 25 °C. The incubation parameter for bacteria was carried out at 37 °C for 18-24 hours and for fungi at 28 °C for 7 days. Once the incubation period was over, the plates were inspected for a clear zone around the well. This clear zone signifies the antimicrobial effectiveness of the compounds under test. The Zone of Inhibition (ZOI) was calculated in millimeters(mm) and further analyzed [29].

Statistical analysis

The results were evaluated based on the mean of three distinct measurements. Microsoft Excel 2021 was used to compute the averages and standard deviations. Graphical illustrations were generated through the software Graph-Pad Prism 10.0.1.

Results and Discussion



Nutritional analysis

Figure 1. Nutritional composition of pulp and seed oil samples of *D. butyracea.*

The seeds were found to have higher protein and fat contents (12.1 ± 0.017 and 38.29 g/100g) compared to the pulp (6.40 ± 0.002, 20.23 g/100g). However, the carbohydrate content in the seeds (0.70 ±0.017 g/100g) was lower than that of the pulp (4.05 ± 0.001 g/100g). Similarly, the pulp contained more ash (6.45 g/100g) than the seeds (2.70 g/100g). Figure 2 displays the nutritional composition of *D. butyracea*. Both the pulp and seed oil contain protein, carbohydrates, fat, and ash.

Phytochemical analysis

The phytochemical contents in the pulp were found to be higher than in the seeds. The Total Phenolic Content (TPC) in the seeds was found to be 486.08 ± 0.006 mg GAEs/100 g, while TPC in the pulp was evaluated to be 182.26 ± 0.001 mg GAEs/100 g. The vitamin C content in the pulp and seeds was found to be comparable (20.70 ± 0.002 and 19.08 ± 0.005 mg AA/100 g, respectively). Additionally, trace amounts of carotenoids, β-carotene, and lycopene were detected in both of these samples (**Table 1**).

Table 1. Phytochemical contents in *D. butyracea* pulp and seed methanolic extracts

Parameter	Methanolic extract	
	Pulp	Seed
Total Phenolic Content (mg GAEs/100 g)	486.08 ± 0.006	182. 26 ± 0.001
Vitamin C (mg AA/100 g)	20.70 ± 0.002	19.08 ± 0.005
β-carotene (mg carotenoids/g)	1.06	0.29
Lycopene (mg carotenoids/g)	0.25	0.03

* The P values for the observed differences in composition between pulp and seed were 0.029 for phenolic, 0.57 for vitamin C, 0.045 for β-carotene, and 0.037 for lycopene. These results indicate that the pulp had significantly higher levels of phytochemicals except for vitamin C, where there was no statistically significant difference.

DPPH radical-scavenging activity

The DPPH radical scavenging activity was assessed. This was effectively done for both the standard ascorbic acid as well as the samples. The EC_{50} value, indicative of the 50% RSA, was calculated based on the DPPH radical scavenging activity. The EC_{50} of the pulp and seed was found to be 2207 ± 0.01 and 1841.05 ± 0.77 µg/mL, respectively.

Antibacterial and antifungal activity of fruit extracts

Methanolic extracts of chiuri were evaluated for antibacterial activity at concentrations of 100, 75, 50, and 25 mg/mL. Unfortunately, the methanolic extracts of *D. butyracea* did not show significant antibacterial activity against either Gram-negative or Gram-positive bacteria, as summarized in **Tables 2** and **3**. In comparison, the antibiotic standard, chloramphenicol (30 mcg), exhibited (ZOI) measurements of 23 mm resistant to *E. coli*, 25 mm resistant to *S. Typhi*, 19 mm resistant to *K. pneumoniae*, and 24 mm resistant to *E. faecalis.*

According to the study, the plant extracts demonstrated greater effectiveness against fungi when compared to bacteria. Both the pulp and seed extracts exhibited antifungal activity against *C. albicans*, whereas the positive control, itraconazole (10 mcg), produced a 22 mm ZOI (**Figure 3**). As for the negative control, DMSO was utilized and did not yield any ZOI against any of the organisms. Seed extracts of *D. butyracea* showed the highest ZOI in comparison to the pulp (**Table 3**).

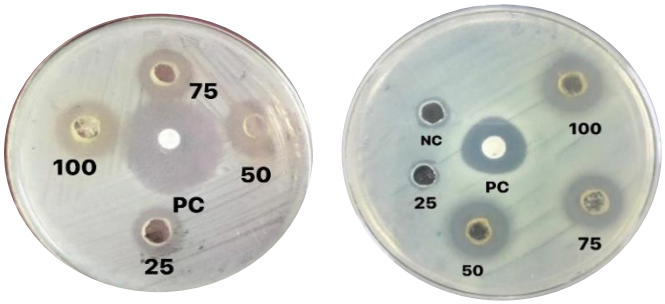


Figure 2. Zones of inhibition as exhibited by the *D. butyracea* seed and pulp extract at 25, 50, 75, and 100 mg/mL concentration against *C. albicans*

Table 2. Zone of Inhibition (in millimeters) of plant extracts against Gram-negative bacteria

Sample	Conc (mg/mL)	Zone of Inhibition (in millimeters)		
		E. coli (25922)	*S. Typhi* (Clinical isolate)	*K. pneumoniae* (700603)
D. butyracea pulp	25	0	0	0
	50	0	0	0
	75	0	0	0
	100	0	0	0
D. butyracea seed	25	0	0	0
	50	0	0	0
	100	0	0	0
	200	0	0	0

Discussion

The chiuri samples were sourced from the Chepang village in the Raksirang Rural Municipality (RRM), Makwanpur. This region heavily depends on chiuri for sustenance. Previous research conducted in this area highlighted the numerous benefits of chiuri and its derived products, including chiuri butter, honey, and seeds [30]. In Nepal and India, chiuri is a common plant among several tribal groups. Considering chiuri are highly valued multi-use species, local communities are working on preserving them on forest lands. In the early 1990s, the District Forest Officer acknowledged the historical users of these trees by granting them tree certificates. This practice was instituted due to the high regard and value that these trees held within the local community [31]. Champ Kharka (elevation 1450 m) and Kamle (elevation 1000 m) in Makwanpur are well known for their chiuri plants [14].

The seeds of chiuri had more protein and fat levels than its pulp, whilst the pulp had higher amounts of carbohydrates and ash, which is similar to *Viburnum mullaha* Buch.-Ham. Ex D. Don, [32]. Contrary to a study, the samples in our analysis had increased protein, fat, and ash contents, while a significantly higher amount of carbohydrate was reported in their study (81.63%) [33]. In another study on chiuri, the seeds showed a fat content of 40.48 %, which is slightly higher than the present value of 38.29% [34]. The research conducted by Sundriyal and Sundriyal aligns with our findings, indicating a similarity in the vitamin C content present in the chiuri pulp [33]. Another study showed the fat content of seeds was 40.48 %, which is slightly higher than our found value of 38.29% [35]. Our research found that the TPC of the chiuri pulp and seeds were 486.08 ± 0.006 and 182.26 ± 0.001 mg GAEs/100 g, respectively. Interestingly, when we compared with the chiuri tree bark, the TPC was 228.53 ± 0.65 mg GAEs/g, which was significantly lower than that of the fruit pulp.

This observation implies that the chiuri fruit boasts a more substantial concentration of phenolic compounds when contrasted with other components of the chiuri tree [36]. In our research, EC_{50} of pulp and seed oil was 2207 ± 0.01 and 1841.05 ± 0.77 µg/mL, respectively, on DPPH assay. The recorded values exhibited a range of 5.15 to 231.9 µg/mL, surpassing the activity levels observed in a prior study involving samples of bark, leaves, and pericarp, which demonstrated lower activity levels [10]. The antioxidant properties of fruit are significantly determined by the presence of vitamin C and phenolic compounds. It is noteworthy that both the pulp and seeds contain similar amounts of vitamin C, suggesting its uniform distribution within the fruit and its significant contribution to the overall antioxidant capacity. It's intriguing to note that the seeds display a higher TPC and exhibit more potent DPPH radical-scavenging activity when compared to the pulp. This suggests that the seeds may have stronger antioxidant properties. This highlights the potential role of phenolic compounds,

Table 3. Zone of inhibition (in millimeters) of plant extracts against Gram-positive bacteria and *Candida albicans*

Sample	Concentration (mg/mL)	Zone of Inhibition (in millimeters)			
		S. aureus (25923)	*B. subtilis* (6051)	*E. faecalis* (29212)	*C. albicans* (10231)
D.butyracea pulp	25	0	0	0	09
	50	0	0	0	11
	75	0	0	0	12
	100	0	0	0	13
D.butyracea seed	25	0	0	0	10
	50	0	0	0	12
	75	0	0	0	13
	100	0	0	0	16

particularly in the seeds, in enhancing the fruit's antioxidant properties.

Our analysis detected trace amounts of β-carotene and lycopene in our samples. These findings align with similar observations in other fruits, such as *V. mullaha* and *Diospyros malabarica* (Desr.) Kostel [32, 33, 37]. The phytochemical composition and nutritional value of plants are subject to significant influences from various internal and external factors. These factors encompass soil quality, climate conditions, weather patterns, harvest timing, altitude, and the use of fertilizers. The intricate interplay of these elements can profoundly affect the chemical composition and nutritional content of plants, including fruits like chiuri. Understanding these dynamics is crucial for assessing the quality and potential health benefits of plant-based foods [1, 10, 38, 39].

For many centuries, various tribal groups in Nepal have utilized *D. butyracea* for a range of medicinal applications. However, scientific investigations into its nutritional and therapeutic properties remain relatively scarce. The butter, extracted from the seed of this plant and rich in triglycerides and fatty acids such as palmitic acid, oleic acid, stearic acid, and linoleic acid, could be a significant nutrient source [2]. In terms of medicinal applications, these specific compounds could potentially be isolated for pharmaceutical use. For instance, oleic acid has been studied for its potential anti-inflammatory, heart health benefits, and cancer-fighting properties [40–43]. As far as we are aware, there have not been any previous scientific studies examining the antifungal properties of *D. butyracea*. Our study represents a pioneering effort in screening the antifungal activities of this particular plant species. The antifungal activity of *D. butyracea* seed and pulp extracts against Candida albicans may be attributed to specific phytoconstituents. Flavonoids and tannins, known for their ability to disrupt microbial membranes and inhibit microbial enzymes, along with glycosides and terpenoids, which can disrupt fungal cell membranes, are likely contributors [44]. Additionally, the presence of phenolic compounds such as gallic acid and catechin, known for their antifungal activities, further enhance the extract's antifungal properties [44]. Tiwari et al. (2020) documented the antimicrobial properties of various parts of the *D. butyracea* plant, including the bark, flowers, and leaves. However, it is worth noting that their study did not encompass the examination of the pulp and seeds, which distinguishes our research by exploring additional components of this plant's antimicrobial potential [17]. Chhetry et al. (2022) conducted an examination of the antibacterial capabilities of the root, bark, leaves, and pericarp extracts from *D. butyracea* [35]. Our investigation revealed that the methanolic bark extract displayed robust antimicrobial efficacy against the examined bacteria. Additionally, the methanolic extracts of the pericarp exhibited antibacterial activity, albeit exclusively against *S. aureus*. However, it's important to note that none of the extracts demonstrated sensitivity to *E. coli,* which aligns with the study [35]. Our samples did not exhibit antibacterial activity, possibly because the fruit and seed contain fewer bioactive compounds compared to other plant parts [45]. This could be attributed to the possibility that certain plant extracts may contain antibacterial components, but their concentrations may not have been sufficient to exert an effect. Additionally, it is plausible that the solvent used did not adequately dissolve the active chemical constituents [45]. Our extract showed action against only *C. albicans,* and similar conditions were observed with the methanolic extracts of *Arum hygrophilum,* suggesting antimicrobial activity may differ with the type of plant species [46]. The predominant bioactive constituents responsible for significant antibacterial activity were identified as phenolics, alkaloids, flavonoids, triterpenes, and steroids [47, 48]. Further studies are warranted to know the composition that influences the plants to exhibit antifungal, antibacterial, or both properties. It is important to note that the results of this study are based on the analysis of *D. butyracea* fruits from a single harvest and region. While this provides valuable insights into the nutritional and phytochemical traits of these fruits under specific conditions, the generalizability of the results may be limited. Ecological conditions such as soil quality, rainfall, and temperature can significantly impact the nutritional and phytochemical composition of fruits. Additionally, the stage of fruit maturity at the time of harvest can also influence these traits. Therefore, variability in these factors could lead to different results. Future studies could strengthen our understanding by analyzing fruits from multiple harvests, locations, and stages of maturity. This would provide a more comprehensive view of the potential range of nutritional and phytochemical traits of *Diploknema butyracea* fruits.

Impliaction for conservation

Biodiversity conservation*:* The manuscript highlights the ecological significance of the Chiuri tree which is prevalent in the sub-Himalayan regions spanning Nepal, India, and Bhutan. [2, 8]. It emphasizes the need for conservation efforts to protect this medium-sized tree species native to the Himalayan belt of Asia [2]. Given its crucial role in local ecosystems and its multiple uses,

including medicinal, nutritional, and economic purposes [2, 8], conserving the Chiuri tree is essential for maintaining regional biodiversity.

Preservation of Indigenous knowledge: The manuscript underscores the traditional knowledge and practices of tribal communities, such as the Chepang community in Nepal, in utilizing *D. butyracea* for medicinal applications. Conservation efforts should not only focus on the tree itself but also on preserving the indigenous knowledge and cultural practices associated with its use. This includes documenting traditional harvesting methods and sustainable approaches to ensure the tree's long-term survival and the communities dependent on it.

Mitigating anthropogenic strains: The manuscript highlights the threat of extinction faced by *D. butyracea* due to anthropogenic pressures. Conservation initiatives should prioritize addressing these strains, which include overharvesting, habitat degradation, and deforestation. Implementing sustainable harvesting practices and creating protected areas or reserves for *D. butyracea* can help mitigate these threats.

Promoting sustainable utilization: The research reveals the nutritional and phytochemical composition of *D. butyracea*, emphasizing its potential as a valuable resource for local communities [34]. Conservation efforts should also focus on promoting sustainable utilization of the tree's products, such as fruits and seeds, to ensure their availability for future generations while preserving the health of the ecosystem.

Scientific research and monitoring: Further scientific research and monitoring of *D. butyracea* populations are crucial for understanding its ecological role, genetic diversity, and response to environmental changes [49]. This information can inform conservation strategies and help adapt them to evolving conditions.

Community engagement: Engaging local communities, especially those with a historical connection with *D. butyracea*, is essential for successful conservation. Collaborative efforts that involve communities in decision-making processes, sustainable harvesting, and awareness campaigns can strengthen conservation initiatives.

Conservation education: Educating the broader public, policymakers, and stakeholders about the ecological and cultural significance of *D. butyracea* is vital. Conservation education programs can raise awareness and garner support for conservation efforts.

Preserving medicinal and nutritional resources: Given the medicinal properties and nutritional value of *D. butyracea*, its conservation is vital for biodiversity, healthcare, and food security. Protecting this resource can improve local populations' well-being and potentially have broader implications for public health.

In conclusion, the manuscript highlights the ecological, cultural, and economic importance of the Chiuri tree and underscores the urgency of its conservation. Efforts to protect this species should encompass a holistic approach that integrates biodiversity conservation, sustainable utilization, community engagement, and scientific research.

Conclusion

The objective of this research was to evaluate the nutritional, phytochemical, and antimicrobial characteristics of *D. butyracea* fruits. Both the pulp and seeds of the fruit show substantial nutritional value and appear to be a moderate source of phytochemicals and antioxidants. Our current research represents an initial exploration into this plant's antibacterial and antifungal potential. The results suggest that the methanolic extracts of the fruit pulp and seeds display promising antifungal properties, especially against *C. albicans*. The seeds were found to have a high protein and fat content, while the pulp was rich in carbohydrates and phenolic content. The study suggests the potential use of fruit in creating functional foods due to their nutritional potential and medicinal value, including bioactive properties. Additional investigations are imperative, particularly in elucidating the mechanisms underlying its antibacterial and antifungal attributes. These efforts are essential for substantiating the plant's ethnomedicinal applications and advancing the scientific exploration of plant-derived antimicrobial agents, which hold significant potential for safe and productive healthcare solutions.

Abbreviations

AA, Ascorbic Acid; ATCC, American Type Culture Collection; CFU, Colony-forming Unit; DCPIP, 2, 6-dichlorophenolindophenol; DMSO, Dimethyl Sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, median effective concentration; ELISA, Enzyme-Linked Immunoassay; GAEs, Gallic Acid Equivalent; $HClO_4$, Perchloric Acid; MHA, Mueller Hinton Agar; NB, Nutrient Broth; PDB, Potato Dextrose Broth; RPM, Rounds per Minute; RSA, Radical Scavenging Activity; TPC, Total Phenolic Content; ZOI, Zone of Inhibition.

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Author's contributions

PD and MAS both authors have equal contribution on this project. PD participated in study design, methodology, laboratory work, data analysis, interpretation, and manuscript writing. MAS contributed to study design, methodology, data analysis, interpretation, manuscript writing, and editing. SB contributed to conceptualization, methodology, laboratory work, data analysis, data interpretation, and manuscript writing. GS assisted in conceptualization, sample collection, and manuscript writing. BK participated in data interpretation and manuscript writing. KR aided in laboratory work, data analysis, data interpretation, and manuscript writing. SE provided revisions and edits. RC contributed to the manuscript writing. DRJ supported the study design. LRB contributed to conceptualization, study design, and revisions.

Conflicts of Interest

There are no conflicts of interest to declare for this research study.

Data Availability

The data used to support the findings presented in this article are included within the article itself. For any further inquiries or requests regarding the data, please feel free to contact the corresponding author, and they will address your queries upon reasonable request.

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