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Pharmacognostic Exploration, Formulation and Evaluation of *Caesalpinia bonduc* seeds syrup and candy formulation

Research Article

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Abstract

The pharmacognostic exploration and formulation development of *Caesalpinia bonduc* seeds for anthelmintic activity. *Caesalpinia bonduc*, a member of the Fabaceae family, is traditionally used in various indigenous systems of medicine for its diverse therapeutic effects. The present study focuses on the detailed pharmacognostic investigation of the seeds, including their morphological, microscopic, and physicochemical properties. Formulation development efforts involved creating herbal formulations incorporating *Caesalpinia bonduc* seed extracts, followed by an evaluation study. The formulation of herbal syrup and herbal candies containing *C. bonduc* not only offers a practical and enjoyable method of ingestion but also paves the way for the incorporation of traditional medicinal practices into contemporary lifestyles. These formulations enhance the compliance of pediatric patients towards anthelmintic medication. The findings emphasize the need for further clinical studies to confirm the safety and efficacy of *Caesalpinia bonduc* in treating helminthic infections, which could lead to the development of novel, affordable treatments for worm-related diseases.

Keywords: *Caesalpinia bonduc*, Anthelmintic activity, Herbal formulation, Phytochemical screening.

Introduction

Medicinal herbs have been used by humans for centuries as a natural remedy for various ailments and disorders. Numerous pieces of evidence, especially through scientific studies, illustrate the immense potential of medicinal plants utilized in various traditional systems (1). Medicinal plants have emerged as a major problem in the world due to a lack of data on the safety and efficacy of medicinal plants used in treatment. The plant *Caesalpinia bonduc* (Family Caesalpiniaceae) is a medicinally important, it is wild, thorny Dicotyledon plant distributed in hotter parts, coastal areas, Deltaic, eastern, western, southern parts of India, and in other Tropics, subtropics of the World (2). The plant has been reported to possess anxiolytic, antinociceptive, antidiarrhoeal, antidiabetic, adaptogenic, anthelmintic, antiestrogenic, anti-inflammatory, antimalarial, antimicrobial, antifungal, antispasmodic, antioxidant, antiproliferative, antipsoriatic, antitumor, larvicidal, muscle contractile, hepatoprotective, anticonvulsant, and antifilarial activities (3). *Caesalpinia bonduc* is popularly known as African nutmeg or fever nut. The kernel obtained from the seed is a popular condiment used as a spicing agent in both African and continental Food in Nigeria (4). They are mostly used as a condiment and flavouring agent. In powdered form, it acts as a stimulant and relieves constipation. The seed is bitter but has no toxic effect on the human body when it's consumed. The root of the plant is used in the treatment of fever, cough, and asthma, while the leaves have great value in the treatment of elephantiasis, intestinal worms, and fever (5).

Figure 1:

***Caesalpinia bonduc* seed**	***Caesalpinia bonduc* plant**	***Caesalpinia bonduc* plant**
		

Taxonomic Classification

- Kingdom: Plantae
- Phylum: Magnoliophyta
- Division: Magnoliopsida
- Class: Angiospermae
- Order: Fabales
- Family: Fabaceae (or Caesalpiniaceae)
- Genus: *Caesalpinia*
- Species: *bonduc*

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Botanical description

Caesalpinia bonduc is characterized by its bitter taste and evergreen foliage. It has deep taproots and a hard, woody stem. The leaves are bipinnately compound, elliptical to ovate, and arranged alternately along the stem. The leaf color is green, with a glossy surface. The plant produces dicot seeds and has a characteristic odor (6).

Synonyms

Caesalpinia bonduc in Hindi called Kantkarej, Kanti Karanja, or Sagargota; in English, it is referred to as Fever nut, Bonduc nut, Nicker nut, or Nicker seed; and in Marathi, it is known as Gajaga (3).

Methods and Materials

Collection and Authentication of Plant Material

Seeds of *Caesalpinia bonduc* were gathered from a natural habitat of the Sewagram region, and the sample was authenticated by the Botanical Department of RTMNU Nagpur University, receiving the specimen number 104217.

Preparation of Plant Extract

The seeds underwent a thorough washing process, were subsequently air-dried at ambient temperature for 7 to 10 days, and were then subjected to pulverization using a mechanical grinder. Seeds were first defatted with petroleum ether in a Soxhlet apparatus and then extracted with a Hydroalcoholic extract (ethanol:water = 70:30 v/v) through sonication. The [ss3] [JD4] resultant extract was concentrated *via* a rotary evaporator and subsequently desiccated to yield a semi-solid mass. The extract was preserved in an airtight container maintained at a temperature of 4°C.

Figure 2. Seeds outercoat

Figure 3. Seeds

Figure 4. Seeds kernel

Figure 5. Seed powder

Pharmacognostic Evaluation

Organoleptic Characteristics like shape, size, color, surface characteristics, and odor of seeds were studied (Table 3).

Physicochemical Parameters

The physicochemical parameters, such as Total ash, acid-insoluble ash, water-soluble ash, extractive values, Loss on drying, swelling index, and foaming index, were studied (Table 3) (7).

Phytochemical Evaluation

The bioactivity of the herbal product was determined by the phytoconstituent present in it. The hydroalcoholic extract of *Caesalpinia bonduc* seed was screened to determine the presence of phytoconstituents by using various chemical tests as per standard procedures (Table 4) (7).[ss5] [JD6]

The confirmatory qualitative phytochemical analysis of plant extracts was conducted to ascertain the predominant classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts in accordance with standard procedure (7).

- **Protein Test: Biuret Test:** Two drops of 0.1% copper sulphate solution and 10% sodium hydroxide solution were added to the test solution, and the production of a violet or pink color was monitored.
- **Test for Free Amino Acids: Ninhydrin Test:** This test solution forms a purple color when it is heated with a 0.2% solution of Ninhydrin, indicating the presence of free amino acids.
- **Test for Carbohydrate: Benedict's test:** A small amount of Benedict's reagent (an alkaline solution containing cupric citrate complex) was added to the test solution, which was then boiled in a water bath. The formation of a reddish-brown precipitate indicated the presence of carbohydrates.
- **Glycoside test (Salkowski's test):** 2 ml of concentrated H^2SO^4 acid was added to the aqueous plant crude extract. A reddish brown color formed which indicated the presence of the steroidal aglycone part of the glycoside.
- **Test for Tannins:** Approximately 200 mg of the plant extract was subjected to boiling with 10 mL of distilled water; subsequently, 0.1% Ferric chloride was introduced to the mixture, which was then scrutinized for the development of a blue-black coloration, thereby indicating the presence of tannins.
- **Test for Alkaloids:** The plant extract was initially dissolved in 100 mL of water, filtered, and subsequently heated in a water bath with 2 mL of the filtrate and three drops of 1% HCl. This mixture was used for the following chemical test.
- **Meyer's test:** To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of cream color precipitate indicated the presence of alkaloids.
- **Dragendroff's reagent test:** 2 mL of extract was heated with 2% H^2SO^4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.
- **Test for Saponins:** Approximately 0.5 milliliters of the extract was mixed with 5 mL of distilled water and mixed. Then, foam formation indicates the presence of saponins.
- **Test for Flavonoids:** Two hundred milligrams of the botanical extract was combined with 10 mL of ethanol and subsequently subjected to filtration. Following this, 2 mL of the filtrate was mixed with concentrated hydrochloric acid and a magnesium ribbon. The emergence of a pink or red color signifies the existence of flavonoids.
- **Test for Steroids:** Approximately 1 mL of the crude extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid; the formation of a bilayer (characterized by a red upper layer and a greenish lower layer) indicates the presence of steroids.
- **Test for Terpenoids:** The assessment of terpenoids was conducted by observing the development of a reddish-brown coloration during the test for terpenoids, which involved the combination of 0.5 mLof the crude extract with 2 mL of chloroform.

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Formulation Development

The *Caesalpinia bonduc* seed shows anthelmintic activity (8). The primary goal of this study was to create a formulation with anthelmintic activity that is appropriate for use in children. The pediatric patient facing problems taking bitter drugs and swallowing, so attempts were made to mold this into a candy and syrup formulation.

Herbal Syrup

Table 1: Composition of *Caesalpinia bonduc* syrup

Ingredients	Quantity	Figure 6: *C. bonduc* syrup
Caesalpinia bonduc seed extract	2 gm	
Sucrose	66.7gm	
Coloring Agent	5 mg	
Flouring Agent	2 to 3 drops	
Water	q.s up to 100ml	

Preparation Method

Seeds of *Caesalpinia bonduc* collected, cleaned, and dried. Seeds were ground and pulverized, and extracted by Soxhlet extraction. Seeds were first defatted with petroleum ether later extracted by hydroalcoholic Solvent (ethanol: water) (20:80).

Preparation of simple syrup IP

66.7 g of sucrose was dissolved in sufficient distilled water to obtain 100 ml of concentrated simple syrup. The solution was boiled, cooled, filtered, and the simple syrup was used as the vehicle (9).

Preparation of (medicated) *Caesalpinia bonduc* syrup

The powdered *Caesalpinia bonduc* extract was added to the prepared syrup base and heated until the volume was reduced to half, cooled and filtered.

Preparation of final syrup

Caesalpinia bonduc extract syrup should be added to the sugar syrup by simple stirring to obtain a uniform and consistent syrup. Coloring agents and flavoring agents were incorporated into the mixture. The resultant syrup was then transferred into an amber-colored bottle, securely sealed, and stored in a cool, dry environment. The final syrup formula is stipulated in Table 1.

Evaluation parameters

- Organoleptic study: The organoleptic attributes, including color and odor of the formulation, were evaluated through visual inspection Table 5.
- Determination of pH: Take 10 mL of the final syrup in the volumetric flask and make up the volume to 100 mL with distilled water. The pH was measured by using a digital pH meter.
- Determination of viscosity: The Viscosity of syrup can be determined by using an Ostwald viscometer. The determination of viscosity was executed employing the following formula: Density of the syrup (d_s) multiplied by the time required for the syrup (t_s) to flow, divided by the density of water (d_w) and the time required for water to flow (t_w).
- Determination of density: The assessment was conducted utilizing the formula delineated below. The Density of the liquid under examination (syrup) is defined as the weight of the syrup divided by the volume of the syrup, represented mathematically as w_s/v_s.
- Determination of specific gravity: The specific gravity of the liquid under examination (syrup) is expressed as the weight of the syrup divided by the weight of water, denoted as w_s/w_w.

Stability testing involved the assessment of the prepared herbal syrup under accelerated temperature conditions. The final syrup was allocated into culture tubes and subjected to accelerated temperatures at 4°C, room temperature, and 47°C, respectively. The samples underwent evaluation for all physicochemical parameters, turbidity, and homogeneity at intervals of 24 hours, 36 hours, and 72 hours to monitor any potential alterations (10).

Herbal Candy

Table 2: Composition of *Caesalpinia bonduc* candy formulation

Ingredients	Quantity per candy	Figure 7: *C. bonduc* candy
Sugar	1.2 g	
Drug (hydroalcoholic extract)	120 mg	
Lemon juice	0.4 mL	
Colouring agent	0.5 mg	
Flavoring agent (cardamon)	0.1mg	
Propyl Paraben	0.02%	
Water	q. s	

Preparation method

The sugar was dissolved in a small volume of water and subsequently heating the mixture until complete dissolution occurred, resulting in a brownish hue. Extract was added to the resultant sugar solution. Furthermore, a small amount of lemon juice was integrated into the preparation to prevent excessive boiling of the sugar solution. Subsequently, beetroot juice was introduced as a coloring agent, while cardamom powder was added as a flavoring agent. Preservatives are incorporated into the blend to enhance its longevity. The concocted mixture is then transferred into a mold and permitted to cool at ambient temperature until it solidifies. Upon cooling, it is imperative to store the product appropriately at the designated temperature (Table 2) (11).

Evaluation parameters

- Physico-chemical parameters: The evaluation of curcumin candies was conducted based on various physicochemical parameters, including color, odor, and taste (Table 6).

- Measurement of pH: The candies were introduced into a 100 ml flask containing 100 ml of distilled water and subjected to sonication for approximately fifteen minutes, after which the pH was determined utilizing a digital pH meter.
- Hardness: To acquire the values pertinent to the candy, the force (N) requisite for the rupture of the candy was quantified utilizing the hardness tester (Pfizer Hardness Tester). Ten candies corresponding to each batch were employed, allowing for the computation of their average breaking force.
- Thickness: The thickness and diameter of the formulated candy were assessed using Vernier calipers. Herbal candies were designed to maintain a uniform thickness to ensure optimal dissolution within the oral cavity.
- Weight Variation: The formulated lozenges underwent an evaluation for weight uniformity, wherein both collective and individual weights of ten lozenges were determined. The average weight was subsequently derived from the combined weight, and the weight of each lozenge was compared against this average to ascertain compliance with permissible limits.
- Friability: The Roche friability test apparatus was employed to ascertain the friability of the lozenges. Ten pre-weighed lozenges were introduced into the apparatus, which was subjected to 100 revolutions, after which the lozenges were reweighed.
- Moisture Content: The sample was weighed and subsequently pulverized in a mortar. One gram of the sample was then measured and placed within desiccators for 24 hours. Following this period, the sample was weighed again. The moisture content was determined by subtracting the final weight from the initial weight of the lozenges.
- Stability Test: A physical stability assessment of the Candy was performed under varied temperature conditions of 2°C, 25°C, and 37°C over four weeks. The Candies exhibited physical stability across the different temperatures, specifically at 2°C, 25°C, and 37°C, throughout the four-week duration (11-13).

Results

In the present study, efforts were made to formulate and evaluate the *Caesalpinia bonduc* herbal syrup. The formulated syrup passes all physicochemical and phytochemical parameters, and the formulation can be further used for anthelmintic activity.

Phytochemical Evaluation

The results of the phytochemical study were presented in Table 4. Phytocompounds are highly present in the hydroalcoholic extract than petroleum extracts. Among all the phytocompounds, alkaloids, flavonoids, polyphenols, saponins, steroids, and tannins show higher concentrations in the hydroalcoholic extract. The hydroalcoholic extract shows the presence of amino acids, carbohydrates, alkaloids, flavonoid glycosides, proteins, polyphenols, and tannins (7).

Table 3: Physicochemical parameters of *Caesalpinia bonduc* seeds

Sr. No.	Parameter	% Values (w/w)
A.	**Ash Value**	
1	Total ash value	33.47
2	Acid insoluble ash value	7.86
3	Water soluble ash value	18.92
4	Sulphated ash value	34.08
B.	Extractive value	
5	Petroleum ether	2.42
6	Chloroform	13.45
7	Ethanol	7.30
8	Water	15.37
9	Moisture content	31.5
C.	Swelling index	5.64
D.	Foaming index	166.67

(% w/w = Percent weight by weight)

Table 4: Phytochemical screening of *Caesalpinia bonduc* seeds

Sr. No.	Phytoconstituents	Petroleum ether extract	Hydroalcoholic extract
1	Carbohydrates	-	+
2	Amino acids	-	+
3	Proteins	-	+
4	Tannins	-	+
5	Flavonoids	-	+
6	Terpenoids	-	-
7	Triterpenoids	-	-
8	Alkaloids	-	+
9	Saponin	-	+
10	Polyphenols	-	+
11	Glycosides	-	-
12	Sterols	+	-

(-) = Negative test; (+) = Positive test

Table 5: Evaluation parameters of herbal syrup

Evaluation Parameters	Results
Colour	Yellowish
Odour	Lemon like
Taste	Sweet
pH	6.2
Specific Gravity	1.06 gm
Density	1.05 gm/ml
Viscosity	1.614 Cps

Table 6: Evaluation parameters of herbal candy

Evaluation Parameters	Results
Colour	Reddish brown
Odour	Lemon like
Taste	Sweet
pH	5.6
Thickness	12.57 ± 0.5
Hardness	6.5 ± 0.25
Friability	0.31± 0.07
Weight Variation	7.26 ± 0.078
Moisture content	0.8%
Stability	Stable at 2°C, 25°C, and 37°C

Discussion

The Caesalpinia bonduc seed shows anthelmintic activity. The primary aim of the present investigation was to formulate and evaluate the polyherbal syrup and polyherbal candy of the hydroalcoholic extract of *Caesalpinia bonduc* seeds used as an anthelmintic agent for paediatric use. This research examines the formulation and evaluation of Polyherbal candies that do not presently exist in the commercial marketplace. The fundamental aspects concerning herbal medicines encompass purity, safety, potency, and efficacy for consumers. Consequently, the standardization and quality control of herbal pharmaceuticals and their raw materials are invariably essential before formulation. The seeds of *Caesalpinia bonduc* exhibit anthelmintic properties (14). The principal objective of the current study was to formulate a preparation that is suitable for pediatric application while eliciting anthelmintic activity. Pediatric patients often encounter difficulties in ingesting bitter medications and swallowing; thus, it was transformed into a syrup and candy formulation for better compliance.

Conclusion

In conclusion, the integration of *C. bonduc* in confectioneries and syrups presents substantial potential for improving adherence of pediatric patients to anthelminthic preparations. *C. bonduc* functions as an antioxidant, serving as an anthelminthic agent that bolsters the body's innate defense mechanisms against pathogenic infections. The formulation of herbal candies containing *C. bonduc* not only offers a practical and enjoyable method of ingestion but also paves the way for the incorporation of traditional medicinal practices into contemporary lifestyles. Further empirical investigations are essential to clarify the specific mechanisms of action, optimal dosages, and the long-term implications of *C. bonduc* in confectionery preparations. Overall, the application of *C. bonduc* in candies signifies a promising domain of inquiry and innovation, presenting a natural and palatable dosage form as an anthelminthic. Furthermore, it underscores the extensive potential of botanical substances in the advancement of novel therapeutic agents characterized by improved efficacy and minimal side effects.

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