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Research Article

Formulation and evaluation of herbal paediatric edible jelly of Breynia vitis-idea for helminthic infections

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Aim and Background: This study delves into the exploration of natural anthelmintics to address helminthiasis, a widespread infectious disease caused by parasitic worms. Leveraging the historical use of plants in phytotherapy, the research focuses on the ethanolic extract of leaves of *Breynia vitis-idaea* for its potential anthelmintic properties. **Methodology:** The investigation involves phytochemical screening, earthworm bioassays, and the formulation of a child-friendly herbal jelly. **Result:** The study reveals promising anthelmintic efficacy in ethanolic extract of leaves of *Breynia vitis-idaea*, particularly at concentrations exceeding 40 mg/mL. The formulated herbal jelly, with Batch F3 exhibiting the desired attributes, offers an appealing solution for parasitic infections in children. The dark green jelly, with a pleasant odor and thick consistency, proves to be a viable alternative to traditional anthelmintic drugs. **Conclusion:** The study underscores the global shift towards herbal remedies, driven by their natural origin and minimal side effects. In regions with limited access to modern healthcare, the reliance on traditional practitioners and medicinal plants becomes crucial, emphasizing the need to integrate traditional wisdom with scientific validation. As the world faces evolving health challenges, embracing and further exploring the potential of herbal interventions is imperative for resilient and sustainable healthcare systems. The results of this study add to the growing body of evidence that herbal alternatives to conventional anthelmintic drugs are better, especially when it comes to genetic resistance. The study also supports using herbal remedies in healthcare.

Keywords: *Breynia vitis-idaea*, Anthelmintic activity, Helminthiasis, Jelly.

INTRODUCTION

The historical use of plants for medicinal purposes, encapsulated by phytotherapy, stems from the rich variety of secondary metabolites they contain, making chemical diversity a central focus in natural products research for effective ailment treatments [1]. Helminthiasis, a prevalent infectious disease affecting a large global population with poor personal and environmental hygiene, involves parasitic worms causing various health issues. Anthelmintic drugs like Albendazole, Mebendazole, Ivermectin, and piperazine citrate have been crucial in combating these parasites, yet genetic resistance poses recent challenges [2]. Hence, exploring alternative strategies, particularly natural anthelmintics, becomes imperative [3].

The prominence of herbal drugs, owing to their natural origin and minimal side effects, is increasing globally [4-6]. In many developing nations, reliance on traditional practitioners and medicinal plants underscores the importance of herbal formulations in healthcare [7,8]. Controlled experimental studies, verifying and quantifying plant activities, include numerous herbs like *Azadirachta indica*, *Allium sativum*, *Chenopodium album*, *Cucurbita pepo*, *Capsicum annuum*, *Curcuma longa*, *Mentha piperita*, *Saraca asoca*, *Ferula asafoetida*, and *Eugenia caryophyllus*, contributing significantly to understanding herbal remedies for parasitic infections [9].

Despite various herbal anthelmintic products in the market, their unappealing taste and odor limit acceptance. Edible jellies, with favorable sensory properties, offer a convenient and acceptable administration method, especially for children. To address this and treat conditions naturally, edible jellies with herbal extracts can be an effective solution.

Breynia vitis-idaea, a non-toxic plant traditionally used as food in parts of Karnataka, India, has various therapeutic compounds [10,11]. Recent studies revealed its anti-hypoglycemic, anti-hypolipidemic, and anti-cancer activities. With known antioxidant properties, the plant contains compounds like 6-O-benzoyl arbutin and breynioside B. However, no previous reports exist on its anthelmintic activity. Given its composition of saponin glycosides, tannins, phenols, and flavonoids, the leaves may possess anthelmintic properties [11-13]. This study investigates the anthelmintic activity of ethanolic leaf extract of *Breynia vitis-idaea* (ELBV) and formulates and evaluates an edible jelly for treating helminthic infections, particularly in children.

Figure 1. *Breyniavitis- idaea plant*

MATERIALS AND METHODS

Collection of Plant

Breynia vitis-idaea leaves were collected from the local areas of Mangalore, Dakshina Kannada Karnataka in June 2023 and authenticated by Dr. Siddaraju M.N. Assistant Professor and Research Guide, Department of Botany, University College Mangalore, Mangalore.

Plant extract

Fresh raw leaves of *Breynia vitis-idaea* were gathered, air-dried for 20 days in the shade, and subsequently ground into a powder. This powdered form was then securely stored in an airtight container. The extraction of the powdered leaves was carried out utilizing the Soxhlet apparatus, employing ethanol as the solvent. About 50g of the powdered material was introduced into the Soxhlet apparatus, and 300ml of ethanol was incorporated into the process. The extraction process involved 17 cycles, yielding the extract, which underwent subsequent filtration. The filtrate was then subjected to evaporation of the solvent at 60°C in an electric water bath, resulting in the formation of a cohesive, sticky mass. The percentage yield was calculated based on the initial quantity of the powder and the mass of the obtained extract [10,14].

Preliminary Phytochemical Investigation

The phytochemical investigation involves the identification of crude drugs based on their phytochemical constituents. This process includes conducting various chemical tests to assess the presence of specific compounds in plants [15,16].

Chemical constituent	Tests	Result
Carbohydrates	Molisch’s test	+
Proteins	Biuret test	-
Steroids	Salkowski reaction	-
Glycosides	Borntrager’s Test	-
a) Anthraquinone glycosides		
b) Saponin glycosides	Foam test	-
Alkaloids	Dragendroff’s test	+
	Hager’s test	+
	Wagner’s test	+
Tannins and Phenolic Compounds:	Lead acetate test	+
	Dilute.HNO3 test	+
Flavonoids	Shinoda test	-

Table 1. *Preliminary Phytochemical screening of Breynia vitis-idaea leaf extract [15-16]*

Earthworm Collection and Authentication

Adult earthworms, African nightcrawler (*Eudrilus eugeniae*) were collected from the Pilikula Vermicomposting unit and authenticated. The worms were washed with normal saline to remove soil and all fecal matter and were used for anthelmintic study. The earthworms 3-5cm in length and 0.1-0.2cm in width were used for all experimental protocols due to their anatomical and physiological resemblance with intestinal roundworm parasites of human beings [17].

Anthelmintic Activity

The anthelmintic activity of formulations containing *Breynia vitis-idaea* was investigated against *Eudrilus eugeniae*. Various concentrations (20, 40, 80, and 100mg/ml) of each formulation was tested in the bioassay, involving the determination of the time of paralysis and the time of death of the worms. Piperazine citrate will be used as the standard reference, and saline water as the control. The assay will be conducted on adult earthworms, specifically, the African nightcrawler (*Eudrilus eugeniae*), chosen for its anatomical and physiological resemblance to the intestinal roundworm parasite in humans, with minor modifications. Earthworms, due to their easy

availability, are widely used for the initial evaluation of anthelmintic compounds in vitro [18,19].

In the experiment, four groups of six earthworms each will be exposed to a 50 ml solution of piperazine citrate and a 50 ml solution of ELBV (at concentrations of 20, 40, 80, and 100 mg/ml each) in distilled water. One additional group will serve as the control. The solutions of piperazine citrate and the ELBV will be freshly prepared before the start of the experiment. Observations will be made for the time taken for the paralysis and death of individual worms. Paralysis will be noted when no movement of the worms occurs, and death will be concluded when the worms lose their motility, followed by the fading away of their body colors [20].

Preparation of the Herbal edible jelly of ELBV:

Various jellies were formulated using gelatin as a gelling agent [21]. Citric acid was employed to regulate the pH [22], while propylene glycol was incorporated to enhance the softness and slipperiness of the jelly [23]. To enhance the overall sensory experience, organoleptic agents were introduced, improving the aesthetic value of the jelly [24]. Methylparaben was utilized as a preservative [25], and honey served the dual purpose of sweetening and acting as a bulking agent in the formulations [26].

Ingredient	Quantity (g)		
	F1	F2	F3
ELBV	5.6	5.6	5.6
Gelatin	2	4	6
Citric acid	1	1	1
Methylparaben	0.01	0.01	0.01
Propylene glycol	3	3	3
Honey	60	60	60
Green S	0.5	0.5	0.5
Peppermint oil	1	1	1
Distilled water	q. s	q. s	q. s

Table 2. *Composition of Herbal jelly [21-26].*

Formulation of Herbal jelly:

Initiate the process by precisely dissolving the weighed components in a specific volume of water. In another vessel, dissolve 60g of honey and transfer it into the mixture containing the accurately weighed ingredients. Dissolve 5.6g of extract in distilled water and amalgamate it with the previously prepared solution. Infuse 1g of Peppermint oil and 0.5g of Green S as flavoring and coloring agents, respectively, ensuring a comprehensive blend. Transfer the resultant concoction into molds to shield it from exposure to the external environment. Finally, envelop the molded jellies in wax paper and securely store them in a cool, dry environment to maintain their quality and longevity [27].

Figure 2. *Preparation of herbal edible jelly using a heating method.*

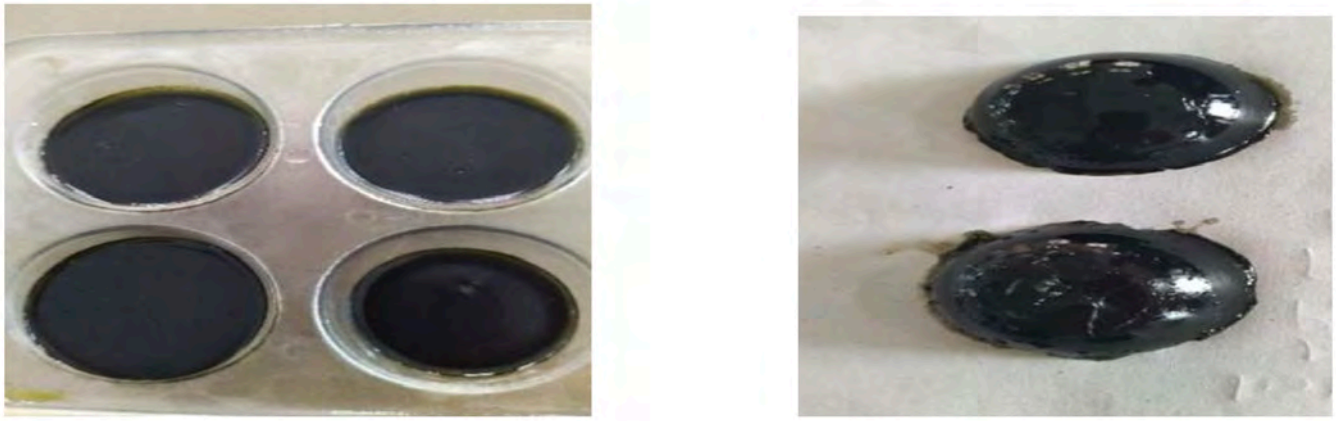

Figure 3. *Herbal edible jelly*

Evaluation Parameter of Herbal Edible Jelly:

1. **Organoleptic properties:** Visual inspection of the consistency and physical appearance of the medicated jelly was done.
2. **pH:** 0.5 g of jelly was dissolved in 50 mL of distilled water to form a 1% solution, and the pH was then measured using a digital pH meter.
3. **Viscosity:** A Brookfield viscometer was used to measure the viscosity at room temperature using spindle number 64 at 1.5 RPM

Statistical analysis

The effects of ELBV were assessed using Mean values and Standard Error Mean as outcome parameters. The analysis of variance (ANOVA) technique was employed to compare the results of the studied medications. Subsequently, data analysis was conducted using ANOVA, followed by the application of Dunnett's test. The significance level was established at *p <0.05. Graph-pad Prism software was utilized for all statistical analyses.

RESULTS

Anthelmintic activity of ELBV in Eudrilus eugeniae.

Test substance	Concentration (mg/ml)	Paralysis (min)	Death (min)
ELBV	20	109.3± 0.80^{ns}	138.0 ± 0.73^{ns}
	40	95.83 ± 0.47*	120.7± 0.71*
	60	73.50 ± 0.69*	118.2 ± 0.47*
	80	66.33 ± 0.98**	102.3 ± 2.78**
Piperazine citrate	25	47.50 ± 0.42***	66.17 ± 0.83***
Control (saline)	0.01	-	-

Table 3. *Observations of Screening of Anthelmintic Activity of ELBV* All Values are denoted as mean ± SEM for n=6, ns= not significant; *p<0.05; **p<0.01; ***p<0.001.

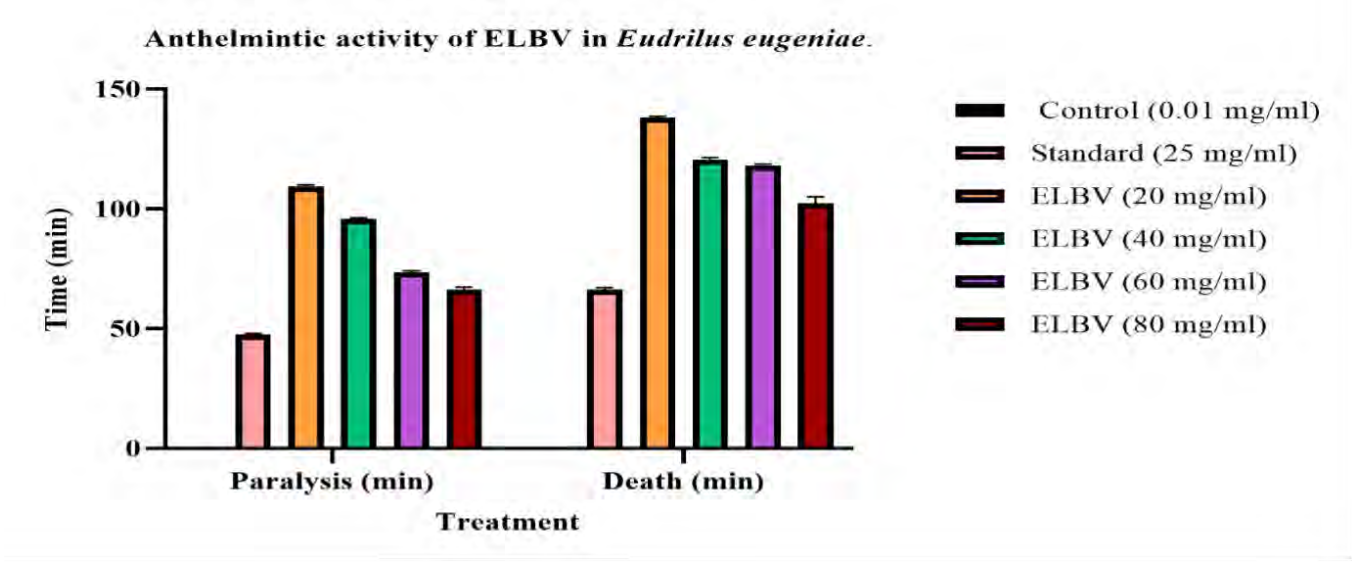

Figure 4. *Anthelmintic activity of ELBV in Eudrilus eugeniae*

Physical evaluation of ELBV Edible Jelly .

Batch	Texture	Consistency	Stickiness
F1	Smooth	Fluid like	Sticky
F2	Smooth	Thick	Sticky
F3	Smooth	Thick	Non-Sticky

Table 4. *Evaluation of Preliminary Batches.*

The batch (F3) which was found with the required characteristics during the preliminary screening was further subjected to evaluation of Appearance, Viscosity, and pH.

Characteristics	Results
Color	Dark green
Odor	Pleasant
Consistency	Thick

Table 5. *Organoleptic evaluation of F3.*

Viscosity and pH determination:

Parameter	Result
Viscosity	137600 cps
pH	4.21± 0.043

Table 6. *Determination of Viscosity and pH.*

DISCUSSION

The increasing prevalence of treatment failures due to genetic resistance in traditional anthelmintic drugs emphasizes the urgency of identifying effective alternatives [28]. Herbal drugs, with their diverse bioactive compounds, offer a promising avenue for overcoming these challenges [29]. This global shift towards herbal remedies is not only driven by their natural origin and minimal side effects but also by the growing recognition of their holistic approach to healthcare [30-31]. In developing nations, where access to modern healthcare may be limited, the reliance on traditional practitioners and medicinal plants becomes even more crucial. This reliance reflects a longstanding cultural connection to nature-based remedies and underscores the importance of integrating traditional wisdom with contemporary scientific validation. As the world grapples with evolving health challenges, embracing and further exploring the potential of herbal interventions becomes imperative for fostering resilient and sustainable healthcare systems [32-34].

Anthelmintic activity of ELBV in *Eudrilus eugeniae* was evaluated, showing a dose-dependent reduction in paralysis and death times. Concentrations of 40mg/ml and above demonstrated significant improvements (*p <0.05), while Piperazine citrate displayed highly significant effects (***p <0.001). The physical evaluation of ELBV Edible Jelly revealed variations in texture, consistency, and stickiness among different batches. Batch F3, meeting the desired characteristics, underwent further evaluation, showcasing a delightful dark green color, pleasant odor, and a thick consistency. Viscosity was measured at 137600 cps, and pH was determined to be 4.21±0.043.

These findings indicate that ELBV possesses promising anthelmintic activity, with the F3 batch of the edible jelly meeting the desired attributes. This formulation is designed to be more child-friendly, providing a better and more enjoyable way to address parasitic infections in children.

CONCLUSION

This study underscores the demand for alternatives to traditional anthelmintic drugs facing genetic resistance. Ethonolic extract of leaves of *Breynia vitis-idaea* shows dose-dependent anthelmintic efficacy, notably in concentrations above 40mg/ml. The physical assessment of ethanolic extract from leaves of *Breynia vitis-idaea* Edible Jelly, especially Batch F3, reveals promising attributes, providing a child-friendly solution for parasitic infections. Overall, these findings highlight the potential of herbal interventions in fostering resilient and sustainable healthcare practices.

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Conflict of Interest: None

Ethical approval: The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Srinivas college of Pharmacy. All the experiments were conducted according to the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA). The study protocol was approved by IAEC, Srinivas College of Pharmacy, Valachil, Mangalore (Ref no. SCP/IAEC/F150/P224/2023) dated 04.08.2023.

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