SEED LONGEVITY AND MEDICINAL PROPERTIES OF *BRUCEA ANTIDYSENTERICA* J.F. MILL. IN ETHIOPIA

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ABSTRACT: Medicinal plants comprise a diverse group of species used traditionally for the alleviating and treatment of human diseases, and they have played an essential role in healthcare systems for thousands of years worldwide. *Brucea antidysenterica* is a well-known medicinal plant in Ethiopia, widely used to treat dysentery and other ailments. However, overexploitation of medicinal plants threatens their long-term survival, highlighting the urgent need for effective conservation strategies. Therefore, this study aimed to evaluate the effects of two storage temperatures (+4 °C and −10 °C) under different storage durations on the seed viability of *B.antidysenterica*. Germination tests were conducted at regular intervals to assess seed longevity during storage. Multivariate analysis indicated no statistically significant reduction in germination percentage ($P < 0.05$) over the storage period. Seeds maintained high viability, with germination percentages exceeding 80% after nine months of storage, including under sub-zero conditions. In parallel, a comprehensive examination of prior findings was conducted to document the medicinal value of the species, using scientific databases such as PubMed, Science Direct, and Google Scholar, with emphasis on studies from Ethiopia published from 2006 -2024. The synthesis of scientific evidence revealed that various parts of the plant are extensively used to treat dysentery, diarrhea, malaria, skin diseases, and other ailments, indicating the importance of conserving the species. The experimental results indicated that *B.antidysenterica* seeds exhibit orthodox storage behavior. However, further studies are recommended to determine the optimal combination of seed moisture content and storage temperature for long-term conservation with certainty.

Key Words: Germination percentage, Germination tests, Orthodox seeds, Storage, Viability

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INTRODUCTION

Medicinal plants are defined as those capable of alleviating or curing diseases due to the various molecules and compounds they contain. They have a traditional use as a remedy in a population or community. Such plants have been used for thousands of years in traditional medicine in many countries around the world. The empirical knowledge about their beneficial effects was transmitted over Centuries within human communities (Khan, 2014). These plants used for thousands of years in traditional medicine in many countries around the world (Marrelli, 2021).

It is estimated that approximately 50,000 plant species worldwide are used in traditional medicinal systems (Chen et al., 2016). In many developing countries, including Ethiopia, traditional medicine remains the primary source of healthcare for nearly 80% of the population (Ekor, 2014; Hamilton, 2004). Moreover, 90% of livestock population in Ethiopia depends on utilization of medicinal plants for primary health care (Kassaye et al., 2007; Yeshiwas et al., 2019).

Teklehaymanot et al. (2006) reported that there are around 800 plant species used in traditional medicine to treat roughly 300 mental and physical disorders. In a study of antiviral properties of Ethiopian plants, Fekadu et al., (2023) identified 387 species, about 6% of Ethiopia's known flora, for which records of use by local communities and traditional herbalists have been documented for the treatment of viral diseases.

The medicinal plants may even be considered as the origin of modern medicine. Medicinal plants are accessible, affordable and culturally appropriate sources of primary health care. Plant-derived compounds have long been and continue to be a major source of molecules for drugs (Salmerón-Manzano et al., 2020). According to Chaachouay and Zidane (2024), in addition to its

employment as folk herbal medicines to treat various disorders, their different natural products have inspired the design, discovery, and development of new drugs. Over 25% of prescribed medicines in developed countries are derived from wild plant species (Hamilton, 2004).

The continued increasing demand of medicinal plants is causing their overexploitation that, subsequently, would result in the extinction of many valuable plants, risking the future benefits and knowledge of human beings. Today, many medicinal plant species are disappearing at a high speed and have reached the fate of extinction or severe genetic loss (Chen et al., 2016). The major threats to medicinal plants include habitat destruction, urbanization, agricultural expansion, investment, road construction, and deforestation (Lulekal et al., 2013). Kloos (2023) also emphasized that overharvesting of medicinal plants and habitat destruction are the major threats to medicinal plant sustainability. In addition to *in-situ* conservation efforts, reproductive materials of medicinal plants should be stored in seed banks for future replanting (Hamilton, 2004). Thus, there is an urgent need to develop and implement conservation strategies of *ex situ* conservation through collection and preservation of seeds.

One of the most basic tools for forest conservation is *ex situ* conservation of seeds in a temperature-controlled environment. Forest tree seeds can be stored in optimum conditions for a long time for later use. Storage of seeds using *ex situ* conservation requires an understanding of the lifespan or longevity of a seed, which is the time period over which it can remain viable. However, not all seeds can be safely stored without a proportional loss of viability. Therefore, investigation if specific storage requirements are needed and for how long seeds can be stored before they lose significant viability is prerequisite for seed handling to obtain preliminary information for their storage. It is imperative to examine the desiccation responses of seeds of unknown storage behavior.

Understanding, documenting and developing conservation strategies are key issues that need to be urgently addressed. *B. antidysenterica*, is one of the most important medicinal plant species. According to Youmbi et al. (2023), *B. antidysenterica* is an African medicinal plant traditionally used to treat cancer, dysentery, malaria, diarrhea, stomach aches, helminthic infections, fever, and asthma. The species is a widely used medicinal indigenous plant in Ethiopia, traditionally employed to treat a variety of ailments. With this background, the current study aims to investigate the impact of storage duration and storage temperature on the viability of seeds of *B. antidysenterica* and its implication for *ex-situ* conservation. In addition, different articles have been reviewed to document the medicinal values and applications of the species. The main objective of this study was to investigate the impact of storage duration and storage environment on the viability of seeds of *B.antidysenterica* and to document medicinal values of the species for the treatments of different ailments and parts used for treatment. The selection of study species was based on the interest in conservation of medicinal plant species.

MATERIALS AND METHODS

Description and geographic distribution of the study species

B. antidysenterica is a plant in the family Simaroubaceae named in honor of a Scottish traveler, James Bruce (1730-1794), who stayed in Ethiopia from 1768 to 1773 and also for its value in traditional medicine for the treatment of dysentery (Jansen, 1981). *B. antidysenterica* is a shrub or smal1 tree to 10(-15) m, often found in regrowth of deforested areas or at forest edges in Montane Forest, evergreen forest, forest margins and montane grass- land at 1650-2800 m (Stannard, B. 1989).

Flowering and fruiting of *B.antidysenterica* continue throughout the year, even during in the dry season (Schmelzer and Gurib-Fakim, 2008; Dagnachew et al., 2023). Jansen, (1981) also reported the plant flowers or fruits all the year round and it is not usually eaten by cattle, sheep or goats, despite the fact that it bears fresh-looking green leaves clustered at the ends of younger branches even throughout the dry season. The plant is traditionally used to treat various diseases in different parts of Ethiopia. It is widely used for treating a number of disorders, including diarrhoea, skin problems and leprosy (Stannard, 1989). The distribution of the plants in Africa and across the flora area of Ethiopia is provided in Table 1.

Seed collection site

Seed collection was conducted in the Choke Mountain Range, near Digotsion town, at the edge of farmland. Collection sites were selected based on the availability of mature seeds at the time of the study. Detailed information on the species, collection altitude, and geographical coordinates is provided in Table 1. All seeds were collected for research purposes under the

standard protocols of the Ethiopian Biodiversity Institute (EBI), a government-recognized institution responsible for national biodiversity conservation. Moreover, the seed samples were collected solely for research purposes and not for storage or further utilization.

Seed collection and processing

Ripe fleshy fruits, characterized by red color, were collected from several individuals immediately after natural abscission. The seeds were collected for research purposes under Ethiopian Biodiversity Institute (EBI)'s institutional mandate. The mature fruits were hand-picked before they had fallen to the ground to avoid the risk of contamination and to collect fruits with healthy seeds. The collected fruits were thoroughly mixed to create a composite sample (ISTA, 2022). The sample was then transported to the EBI, Addis Ababa, on a vehicle in an ambient condition within two days of collection. During transportation, the fleshy fruits were kept in small quantity of small Cotton bags to be well ventilated to avoid seed suffocation and decay following Hong and Ellis, (1996). The cleaning of fruits was conducted upon arrival at EBI. All experiments were subsequently conducted at the Institute. During cleaning, the fleshy fruits were soaked in water to facilitate softening of the pulp. Seeds that float to the top were typically considered non-viable and discarded. The fleshy pulp was removed by rubbing between hands and continuously washing with water (Rao, et al., 2006). The moist seeds were air dried under shade immediately after extraction.

Seed drying, moisture content determination and seed storage

In the laboratory, cleaned and air-dried seeds were placed in a drying room set at 15^{o}C and 15% relative humidity for three weeks. The moisture content of seeds was determined gravimetrically with 5 g of three replications using the low-constant-temperature oven method (103 ± 2^{o}C for 17

± 1h) following the method recommended by Rao et al (2006). Seed moisture content expressed as the wet-weight (wb) computed using the following equation:

$MC\% = \frac{W2-W3}{W2-W1)} X100$; Where, MC moisture content, W1= weight of container; W2 = weight of container and seed sample before drying; and W3 the weight of the container with seed sample after oven drying.

After seed moisture content reduced to desired moisture content level (Table 1), seeds of *B. antidysenterica* were tested for initial germination. The remaining seeds were stored in two cold rooms set at 4^{o}C and -10^{o}C temperatures with sealed aluminum foil bags for consequent germination tests. The seeds were stored for 3, 6 and 9 months.

Germination Tests

After each storage interval, germination tests were conducted in a laboratory at room temperature maintained at 15 to 21^{o}C with 12 h day and 12 h night cycle. Four replicates of 25 seeds per treatment (a total of 100 seeds) were allowed to germinate before the storage of seeds and after each storage interval to determine longevity of seeds at different storage durations. The seeds were periodically tested for their germination percentage at three-month interval for 9 months as recommended by (Hong and Ellis (996), as applied in previous studies by Dagnachew et al. (2023) and Mewded et al. (2022).

Germination of seeds was conducted on plastic boxes of 6 x 18 x 26 cm with 13 randomly equidistant holes at the bottom. The holes are used for air circulation on sand medium moistened with 20 ml of water for 1 kg of sand. The sand was sterilized and the plastic boxes were half

filled in which the seeds were covered with the sand as recommended by Rao et al. (2006). The samples were watered during the whole germination period to maintain the moisture of the sand.

Germination assessments were made on weekly basis. Germination was defined as the emergence of a 2 mm radicle based on visual observation, indicating the potential for the seed to develop into a normal seedling. Germinated seeds were evaluated, counted and removed from the trays in each count. Tested seeds were incubated for up to 60 days in which all experiments continued until no seed germinated for two weeks. The number of germinated seeds was converted to germination percentages.

Medicinal value documentation

Data regarding the medicinal properties and utilized parts of *B. antidysenterica* were summarized from peer-reviewed articles, ethnobotanical surveys, and pharmacological studies published between 2006 and 2024. To document these data, a comprehensive literature search was performed across scientific databases, including PubMed, ScienceDirect, and Google Scholar. The search strategy utilized combinations of keywords such as “medicinal plants of Ethiopia”", “traditional medicine”, and “pharmacological properties of *B. antidysenterica”* with an emphasis on studies undertaken in Ethiopia. The medicinal uses of the *B.antidysenterica* presented in a tabular form (Table 2).

Table 1. Species description, geographical distribution, altitude and geographical coordinates of seed collection **sites**

AR = Arsi, BA = Bale, EW - Eritrea West above 1000m contour TH, GD = Gonder, GJ = Gojjam, HA = Harerge, IL = Illubabor, KF = Keffa, SD = Sidamo, SU = Shewa Upland, TU = Tigray Upland. (Stannard, 1989).

Local Name	Growth Habit	Species Altitudinal Range and Brief Botanical Description	Geographical Distribution Throughout Ethiopia	Geographical Distribution in Africa	Altitude and (Geographical Coordinates) of Seed Collection site	Seed Moisture Content (%) during Storage
Abalo (Amharic)	Shrub or small tree to 10(-15) m.	Montane forest, evergreen forest, forest margins, secondary growth in deforested areas, montane grass-land;1,650-2,800 m.	EW, TU, GD, GJ, WG, SU, AR, IL, KF, SD, BA HA;	Angola, Burundi, Cameroon, Central Afncan Republic, Congo, Ethiopia Kenya, Malawi, Mozambique, Nigeria Ruanda, Sudan	Alt 2732 (10° 53' 03.4"N, 37° 43'49.2" E.)	3.1

Table 2. Summary of Traditional Medicinal Uses of *Brucea antidysenterica*

Study (Plant Material collection area)	Medicinal Uses	Parts Used	Mode of preparation and application	Source(s)
(Lummamie District) east Gojjam zone of Amhara Region	antimalarial effects	Leaves and seeds	extracts of the seed and leaf of *Brucea antidysenterica* and *Ocimum lamiifolium* (Plant parts air-dried at room temperature under shade, without exposure to sun light, and ground into powder)	Kefe et al., 2016
Zegie and Lake Tana monasteries in Northwest Ethiopia Amhara regional state	Dysentery (‘Kuruba’)	Leaf	Juice of leaf is taken orally in the morning	Amuamuta et al., 2015
	Hemorrhoids,	Leaf or fruit	Fruit or leaf powder mixed with milk is taken orally for three days	
	Weight loss, fever, itching, and diarrhea	Leaf	Leaf/fruit powder mixed with honey and fermented for seven days is taken orally until cure	
Guduru District of Oromia Regional state	Used to treat Ascaris	Not mentioned	Ethnobotanical data	Tadesse et al., 2018
Atawi, Wara Jarso woreda, Semen Shoa Zone, Oromia Regional state	wound healing and antibacterial activities	Powder of fresh leaves	Methanol (80%) leaf extract of *Brucea antidysenterica* was fractionated using three solvents; water, n-butanol and chloroform. An ointment containing 2% and 4% of each fraction was formulated and applied to wounds inflicted on rats	Wolde et al., 2022
Bahir Dar rural Kebeles Amhara regional state	To treat Wound	Leaf	The leaf of *Brucea antidysenterica* is pounded, squeezed and then creamed on wounded part until getting recovered	Mazengia et al., 2019

Nensebo District West Arsi Zone of Oromia regional state	Ear infection Hair fungus	Not mentioned	Ethnobotanical data	Abdela et al.,2022
Dega Damot district West Gojjam Amhara regional state	used for the treatment of cutaneous leishmaniasis	extract of seeds	The crude (80% methanol) extract of *Brucea antidysenterica* seeds and its fractions were evaluated for their anti- leishmanial activities against mammalian cells.	Ketema et al., 2023
Wayu Tuka District East Welega Zone Oromia regional state	To treat Ascaris	Fresh leaves	Fresh leaves crushed and mixed with Leaves of Bersema abyssinica Fresen. and cooked With porridge and given for a person in need	Megersa *et al*., 2013
Kembatta Tembaro (SNNPR) of the Southern Ethiopia.	To treat diarrhea	Leaves/Seeds	Buds used to treat Snake bite and leaves and seed used to treat Diarrhea/	Maryo et al., 2015
Gudaya Bila district, East Wollega Zone, Oromia Regional State	Active against all bacterial species	powdered leaves of the plant	The extracts obtained as such were subjected to phytochemical screen-ing and the results of the screening revealed the presence of diverse phytochemicals about the extracting solvents	Guluma et al., 2020
Debre Libanos monastery (9 ◦ 72’ N, 38 ◦ 87’ E)	Kusil (wound) healing		Oral uses water paste beaked –‘Venereal Diseases’ Powder butter paste dressing to treat wound -‘Ekeke’ Nasal Inhaling of Smoke of mixed powder to treat-‘Evil Eye’	Teklehaymanot et al., 2006
Zegie and Lake Tana	wound healing	leaf extracts of	leaf extract aqueous solutions of *Croton marcostachyus*	Mekonnen et al., 2019

	potential	*Croton marcostachyus* and *Brucea antidysenterica*	and *Brucea antidysenterica* were prepared and applied to wound of mice	
Debre LibanosWoreda Central part of Ethiopia	Wound and Skin itching	Leaves	Leaves crushed and mixed with butter to cover the wound	Getaneh and Girma, 2014
Jawi district, located in Northwestern Ethiopia,	To treat Gastritis	Bark and root	Dried stem bark and root part pounded, mixed with water and administered orally	Derso et al., 2024
'Lumame' District east Gojjam zone of Amhara region	Anti-diarrhoeal and antibacterial activities	Roots	The dried crude extract extracted by maceration technique using 80% methanol	Zewdie et al., 2020
published research findings on Ethiopian traditional medicinal plants	for the management of rabies	Fruit Leaf Root	Squeezed and baked with teff flour and given for 3 days [together with *Croton macrostachyus* and *Rumex nervosus*].	Meresa et al., 2017
West region of Cameroon (5° 03′ 60.00" N / 10° 27′ 59.99"E)	antiproliferative activity against cancer cell lines.	Stem and leaves	Phytochemicals isolated from the leaves and stem crude extracts and compounds	Youmbi et al., 2023
Bakouok (Bamenda)	against the	Root and bark	its extract might be considered an	Makong et al., 2019

Germination data analysis

Germination results were analyzed using Microsoft Office Excel (Version 2010). Bar graphs with standard error were used to analyze results of germination under different storage duration and storage environment. The percentage data from the different germination tests were first arcsine transformed to meet the requirement of normality (Zar, 2010). A two-way ANOVA was performed to examine the main effects of storage temperature and storage duration, as well as their interaction, on germination percentage. The effects of storage temperatures and durations on the germination of seeds were evaluated separately using a generalized linear model (GLM) for multiple comparisons between treatments. Adjusted p-values < 0.05, obtained using the Bonferroni–Holm method, were considered statistically significant. Tukey's Honestly Significant Difference (HST) test was used for pairwise comparisons, and significant differences between treatment means.

RESULTS AND DISCUSSION

Storage trial-based germination test

In the initial test, which has been conducted after drying before the storage of seeds, the germination percentage was 97%. For seeds stored at 4°C, the germination percentage decreased steadily over storage periods up to six months compared with seeds stored at -10^{o}C (Fig. 1 and Fig. 2). The germination percentage decreased steadily over storage periods up to six months for seeds stored at 4^{o}C. However, after nine months of storage, seeds maintained a relatively high germination percentage (80%), representing only a slight reduction from the initial value (Figure1). After six months, the germination percentage showed a noticeable reduction in

germination percentage, compared to. initial germination percentage but sustained acceptable viability percentage. In contrast, seeds stored at −10 °C showed a noticeable reduction in germination percentage after three months of storage. However, beyond this initial decline, germination percentage remained relatively stable and was not significantly affected by further increases in storage duration up to nine months (Figure 2).

In general, no significant reduction in germination percentage was observed in *B. antidysenterica* seeds after nine months of storage under either 4 °C or −10 °C storage environment. A two-way ANOVA indicated that there was no statistically significant reduction in germination during storage time for seeds stored at both 4 °C and −10 °C ($F(3, 24) = 1.525$, $p = 0.233$).

Comparing the two storage environments, seeds stored at −10 °C exhibited a relatively higher germination percentage. This higher germination and greater stability of viability with increasing storage duration are characteristic of orthodox seeds. According to (Hong and Ellis, 1996), if no loss in viability is evident during subsequent storage, then the species probably shows orthodox seed storage behavior. The current result revealed that *B.antidysenterica* can be grouped in the orthodox seed storage category. Roberts, (1973) explained that orthodox seeds have enhanced seed longevity at lower temperatures and lower moisture contents, which enhances viability and germination.

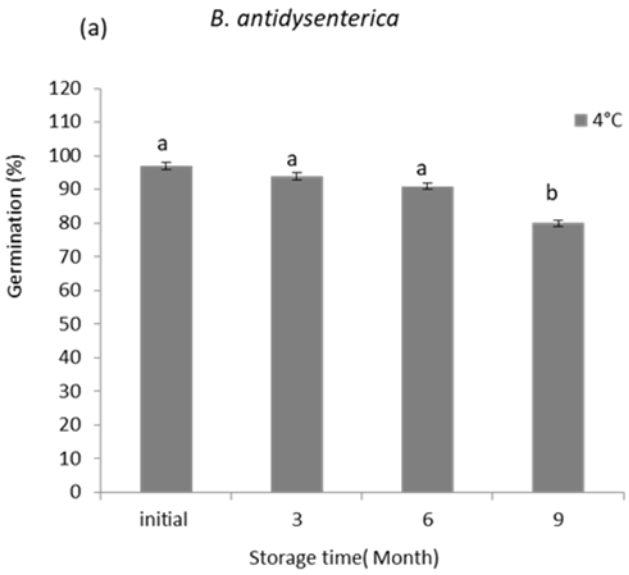

Figure 1. Germination of *B. antidysenterica* stored at 4°C moisture content and over a period of 9 months. Bars with different letters show statistically significant differences.

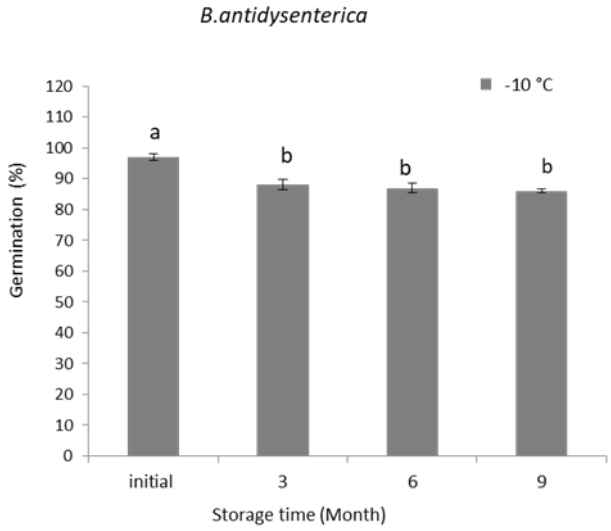

Figure 2. Germination of *B. antidysenterica* stored at -10°C moisture content over a period of 9 months. Bars with different letters show statistically significant differences.

Findings from different researchers also support the principle that lower storage temperatures enhance seed longevity by preserving germination capacity and viability compared with warmer

storage conditions. Several studies (Nyo *et al*., 2019; Hong and Ellis, 1996) have shown that maintaining seeds at low temperatures and low moisture content effectively slows physiological and biochemical deterioration processes, thereby sustaining seed viability over extended storage periods. This pattern has been observed in various species and is well documented in studies such as Mewded et al. (2022) and Dagnachew et al. (2023). Although laboratory conditions differ from those found under field conditions, assessing the effects of storage on germination provides valuable information for ecological restoration and germplasm conservation. Although the storage duration of the present experiment was relatively short (nine months), the findings provide useful guidance for long-term storage of *B. antidysenterica* seeds in a conventional seed bank.

Medicinal values and parts used

The medicinal value of *B. antidysenterica* in Ethiopia is extensive in traditional folk medicine. As its name suggests, it is widely used to treat dysentery and diarrhea, but the species demonstrates a wide range of medicinal properties and has been extensively used in traditional medicine for treating several ailments across several regions of Ethiopia (Yirgu, 2019; Zewdie et al., 2020; Table 2). Numerous researchers indicated that various parts of the plant (roots, leaves, bark and seeds) are traditionally employed against ailments like malaria, leishmaniasis (including cutaneous leishmaniasis), helminthiasis (intestinal worms), wounds and skin complaints (such as scabies, leprosy, and eczema), fever, cancer, weight loss, stomach ache, and as an anti-inflammatory agent (Amuamuta, 2015; Makong, 2019; Wolde et al., 2022 and Ketema et al., 2023).

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated that *B. antidysenterica* seeds maintained high germination percentages over nine months of storage at both 4 °C and −10 °C, indicating no rapid loss of viability during this period. These findings suggest that the seeds of this species can be classified as orthodox, capable of tolerating drying and low-temperature storage at conventional seed bank facilities. Moreover, a review of ethnobotanical literature confirmed that *B. antidysenterica* is widely used in traditional medicine in Ethiopia, particularly for the treatment of dysentery and diarrhea, as well as for malaria, leishmaniasis, intestinal worms, skin diseases, fever, stomach ache, and as an anti-inflammatory agent. The combination of traditional importance and demonstrated seed storage category urges the value of conserving this species both in situ and ex situ. Based on the current findings, further studies should include desiccation screening tests under different temperature regimes and moisture levels, together with longer storage durations, to determine optimal storage conditions for long-term conservation with greater certainty.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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