Review Article

EXPLORING SEED DORMANCY IN MEDICINAL PLANTS – A COMPREHENSIVE REVIEW

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ABSTRACT

This review explores the complex field of studies on medicinal plants during dormancy, aiming to provide a comprehensive overview of research findings and methodologies. Dormancy, a crucial physiological phase in the growth stage of plants, plays a master role in regulating growth, development and survival strategies. In medicinal plants, understanding dormancy is of paramount importance due to its implications for cultivation, propagation and the production of some compounds which are need of hour for pharmaceutical industry. This review focuses on methodologies in dormancy research, elucidating the underlying mechanisms and exploring strategies to manipulate dormancy for enhanced medicinal plant cultivation and utilization. Furthermore, it discusses the challenges in dormancy studies, highlighting the potential for novel approaches and interdisciplinary collaborations to unravel the complexities of dormancy and harness its benefits in medicinal plant research and industry.

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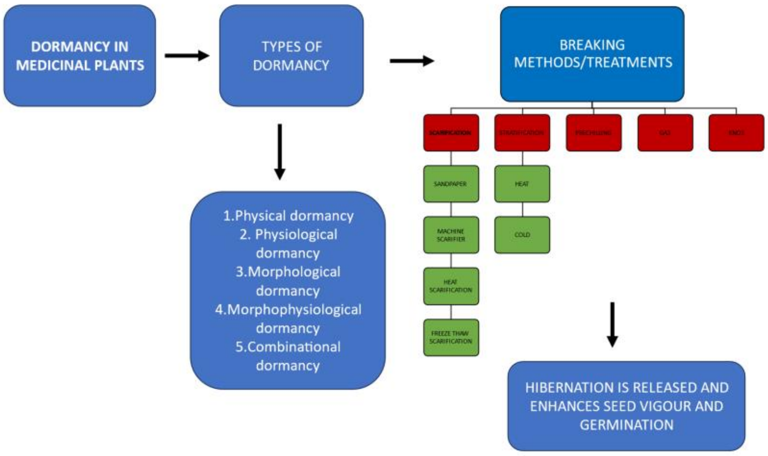
Graphical Abstract



KEYWORDS: Hormones, Physiology, Regulation, Scarification, Secondary Metabolites, Stratification

INTRODUCTION

Therapeutic plants are essential to both traditional and contemporary medicine because they are rich in bio active substances. Further to being used for medical intensions, medicinal plants can supply pharmaceutical businesses with raw ingredients (Abdallah *et al.*, 2023). Nevertheless, issues with seed quality, germination rates and genetic stability arise in the long-term production of therapeutic plants. To tackle these issues and promote the study culture, this analysis seeks to showcase the latest developments in seed technology. Medicinal plants constituting 4.4% of the total worldwide seed market, in which the United States makes up 27%, China 20%, France 8% and Brazil 6%. Medicinal plants are those that are often used to cure and prevent specific diseases and disorders (Zare *et al*., 2011). Plants are the green factories of medication and a vital part of global health. ~~Indigeneous~~Indigenous health care systems are still commonly used in many contexts. The attention on utilizing plant materials as a source of pharmaceuticals for a diverse applications of human needs has increased as a

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result of infectious diseases. These include an insufficient supply of medicine due to population growth, excessive therapy costs, unfavourable effects of different synthetic drugs, and the establishment of drug resistance to frequently prescribed drugs (McDonald, 2000).

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These plants are used as a raw material for the pharmaceutical sector, which has led to their recent major relevance in agriculture, medicine and exports. The majority of medicinal plants have issues in the field with seed quality, especially stand establishment and germination. Techniques for improving seed germination and seedling establishment will improve the performance of medicinal plant production because of their significance in the plant life cycle. Recently, the value of several plants for agriculture and medicine has been discovered in an attempt to save people's livelihoods worldwide (Hassan and Mathesius, 2012).

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Offering solutions to enhance seed germination and seedling establishment will contribute to improved concert in the cultivation of medicinal plants, since the life cycle of plant depends on these mechanisms. Globally, curative plants are a tremendous source of novel pharmaceuticals (Chen *et al.*, 2016). In the United States, over 118 of the top 150 prescription medications are extracted from natural sources, while over 1300 medicinal plants are explored in Europe, with 90% of them being gathered from wild resources (Nalawade *et al.*, 2003). Moreover, approximately 25% of recommended medications in wealthy nations come from wild plant species, while up to 80% of individuals in underdeveloped nations completely rely on herbal remedies for their primary health needs (Balunas and Kinghorn, 2005 ; Chacko *et al.*, 2010). A conservative estimate suggests that the current rate of plant species loss is between 100 and 1000 times higher than the natural extinction rate and approximately one potential significant drug is lost every two years (Cole *et al.*, 2007). The International Union for Conservation of Nature and the World Wildlife Fund report that globally, there are between 50,000 and 80,000 flowering plant species used for medicinal purposes, with about 15,000

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species endangered by expansion due to over harvesting and habitat destruction (Pimm *et al.*, 1995). Despite awareness of this threat for decades, the accelerated loss of species and habitats globally poses an increased risk for the extinction of medicinal plants, especially in countries like Kenya (Heywood and Iriondo, 2003), Nepal (Hamilton, 2004) India (Ross, 2005), Uganda (Larsen and Olsen, 2007), Tanzania (Zerabruk and Yirga, 2012) and China (Chen *et al.*, 2016).Over 50,000 plant species, more than one-tenth of all plant species, are utilized in medications and healthcare items (Srujana *et al.*, 2012). However there are geographical differences in the global distribution of therapeutic plants (Schipmann *et al.*, 2003). With 11,146 and 7,500 species, respectively, China and India have the highest numbers of ~~medicinal~~ plants used in medicine. In these medicinal plants, dormancy signifies a temporary cessation of visible growth, serving as an adaptive mechanism to endure adverse environmental conditions. This dormancy phase notably impacts the production of secondary metabolites, often reducing their synthesis. To improve germination in medicinal plants, knowledge on dormancy, types of dormancy and dormancy breaking treatments are essential. In this context, the information on above points are reviewed hereunder for the benefits of scientific community.

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DORMANCY – THE HIDDEN PULSE OF BOTANICAL VITALITY

Seed dormancy, characterized as the failure of a viable seed to complete germination under right circumstances, is influenced by environmental factors such as light, temperature and the duration of seed storage (Macchia *et al.*, 2001). Seed dormancy is commonly understood as a hindrance to the complete germination of a viable seed even under favorable conditions. However, some literature has reported potential sources of confusion. ~~Ecological studies~~, Fenner & Thompson

(2005) as well as Thompson *et al.*, (2003), for instance, have encountered confusion between seed dormancy and soil persistence, ~~(Fenner & Thompson, 2005; Thompson *et al.*, 2003)~~ stemming from differing views on dormancy, particularly regarding the role of light in either terminating dormancy or inducing germination (Walck *et al.*, 2005).

A formulated dormancy classification system acknowledging the interplay of both morphological and physiological features in seed dormancy was given by Baskin & Baskin ~~,~~ (2021). Expanding upon this framework ~~–~~, Baskin & Baskin ~~(,~~ 2004) introduced a comprehensive classification system comprising five main classes of seed dormancy: Physical, Physiological, Morphological and Combinational dormancy. This hierarchical system, outlined below, further categorizes these classes into various levels and types. Dormancy in seeds is not just escaping the germination; rather, it refers to the seed's characteristic that determines the potential required for germination (Fenner & Thompson, 2005; Vleeshouwers *et al*., 1995). Any environmental issue that alters the conditions which is necessary for germination is considered to be altering dormancy. Moreover, when the seed no longer requires specific environmental cues, it is considered to be nondormant. Researchers have shown that various environmental factors can influence dormancy. For instance, while some suggest that only temperature can alter physiological dormancy following dispersal ~~(~~Krock *et al*., (2002) demonstrated the induction of secondary dormancy in *Nicotiana attenuata* seeds by naturally occurring chemical signals, such as abscisic acid (ABA) and other terpenes found in leachate from litter covering the seeds in their habitat. A seed that is entirely non dormant possesses the ability to germinate across the broadest range of normal physical environmental factors like temperature, water availability, nutrients that are feasible for its genotype (Baskin & Baskin, 2004).

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EXPLORING DORMANCY PATTERNS IN MEDICINAL SEEDS

The exploration of seed germination in medicinal plant species has drawn particular attention from the scientific community, driven by the escalating demand for these plants in the pharmacological industry and the necessity to establish rational crops for herb production (Hassan & Mathesius, 2012; Sajjadi, 2006). In the domain of medicinal plant cultivation, recognizing and addressing seed dormancy is paramount for achieving successful germination and robust crop establishment. By elucidating the dormancy characteristics of various medicinal plant species, cultivators can refine their techniques and harness the full potential of these valuable botanical resources (Penfield & MacGregor, 2017).

Understanding dormancy patterns in medicinal plant seeds is essential for cultivators seeking to optimize germination success and crop yield. Tailoring germination protocols to address specific dormancy types can significantly enhance cultivation outcomes. Additionally, knowledge of dormancy can inform seed storage and handling practices to maintain seed viability over time. Understanding the germination behavior of medicinal plant seeds is fundamental for successful cultivation. One significant aspect to consider is seed dormancy, which can greatly influence germination rates and plant establishment. Here are some of the important species of therapeutic plants that are imposed to different kinds of dormancy.

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PHYSICAL DORMANCY

This type of hibernation arises from water-impermeable layers of palisade cells in the seed or fruit coat, which regulate water movement. Physical dormancy can be broken through mechanical or chemical scarification methods. Examples of species exhibiting this dormancy include *Melilotus* and *Trigonella* (Fabaceae) (Barekat *et al*., 2013). Physical dormancy and its breaking methods for few crops are listed in table 1.

TABLE 1. PHYSICAL DORMANCY OF MEDICINAL PLANTS AND THEIR TREATMENTS

SN	NAME OF THE MEDICINAL PLANT	BREAKING METHODS	REFERENCE
1	Common wormwood (*Artemisia absinthium*)	~~Pre-soaking~~Pre-soaking in distilled water for 8 h	(Mannan *et al*., 2012)
2	Amaranthus (*Amaranthus annus*)	~~Pre-soaking~~Pre-soaking of seeds in distilled water for 6 h	(Kępczyñski *et al*., 2003).
3	Cornflower (*Centaurea cyanus*)	Mechanical scarification until the seed coat is damaged.	(Stankiewicz-Kosyl & Haliniarz, 2023).
4	Saw Palmetto (*Serenoa repens*)	Scarification by sand paper	(Schmalzer & Foster, 2018).
5	St.John's Wort (*Hypericum perforatum*)	Soaking in KNO_3 at 2g/lit for 3h	(Pérez-García *et al*., 2006)
6	Andrographis(or King of Bitters) (*Andrographis paniculata*)	Acid scarification using H_2SO_4 for 4min.	(Kumar *et al*., 2011)
7	Greater Burdock (*Arctium lappa*)	Leaching in fresh water	(Farhoudi *et al*., 2021)
8	Chinese Lantern (*Physalis alkekengi*)	Soaking in GA_3 250ppm for 6h	(Asgari *et al*., 2018)
9	Summer Savory (*Satureja hortensis*)	Soaking in KNO_3 at 2g/lit for 5h	(Aghilian *et al*., 2014a)
10	Securigera (*Securigera securidaca*)	Leaching in fresh water	(Aghilian *et al*., 2014a)
11	liquorice (*Glycyrrhiza glabra*)	Acid scarification for 5 min using HCl	(Aghilian *et al*., 2014a)

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12	Tanacetum (*Tanacetum sp*)	Soaking in KNO_3 at 2g/lit for 3h	(Aghilian *et al.*, 2014a)
13	Valerian (*Valeriana officinalis*)	Cold stratification at 5 °C for 8 days	(Heidari Sureshjani *et al.*, 2022)

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PHYSIOLOGICAL DORMANCY

It is the most common type of hibernation found in seeds of gymnosperms and all major groups of angiosperms. It is especially prevalent in seeds from temperate regions (Bano *et al*., 2021). It can be divided into three groups based on the depth or intensity of dormancy: deep, intermediate and nondeep. Deep dormancy requires more prolonged exposure to cold stratification or other treatments to break dormancy, while nondeep physiological dormancy can be broken with shorter periods of stratification. The varying depths of this dormancy allow plants to stagger germination and protect themselves against unforeseen conditions. Its nuanced nature, with differing depths of dormancy, provides an adaptive advantage for plants in temperate climates with seasonal variations in climate (Zarei-Gavkosh *et al*., 2022). Physiological dormancy ~~has many reasons and background to occur and differs from one to on~~differs from plant to plant, since there are many factors influencing this kind of dormancy (Table 2). ~~Some of them are narrated below in table 2.~~

Table 2. Physiological Dormancy and their breaking treatments

SN	NAME OF THE MEDICINAL PLANT	BREAKING METHODS	REFERENCE
1	Dill (*Anethum graveolens*)	Pre chilling at 5 °C for 6 days	(Bukharov *et al.*, 2021).
2	Colocynth (*Citrulus colocynthis*)	Fluctuating temperatures	(Saberi *et al.*, 2011).
3	Wild sweet William (*Saponaria officinalis*)	Warm stratification	(Li et *al.*, 2020)
4	Ammoniacum (*Dorema ammoniacum*)	Cold stratification at 5 °C for 6 days	(Naseri *et al.*, 2019)
5	Purple cone flower	Prechilling at 5 °C for 6	(Qu *et al.*, 2005)

	(*Echinacea purpurea*)	days	
6	Galbanum (*Ferula gummosa*)	Prechilling at 5 °C for 6 days	(Rahnama-Ghahfarokhi & Tavakkol-Afshari, 2007)
7	Hyssop (*Hyssopus officinalis*)	Prechilling at 4 °C for 4 weeks	(Arves *et al.*, 2013).
8	Tree Mallow (*Malva dendromorpha*)	Stratification (alternating temperatures)	(Aghilian *et al.*, 2014b)
9	High Mallow (*Malva silvestrys*)	Prechilling at 5 °C for 6 days	(Ansari *et al.*, 2016)
10	White Horehound (*Marrubium vulgare*)	Stratification (alternating temperatures)	(Javaid *et al.*, 2018).
11	Lemon Balm (*Melissa officinalis*)	Prechilling at 5 °C for 6 days	(Fahim *et al.*, 2021).
12	Oregano (*Origanum vulgare*)	Warm stratification	(Farashah *et al.*, 2011).
13	Flea Plant (*Plantago psyllium*)	Prechilling at 5 °C for 6 days	(Aghilian *et al.*, 2014a)
14	Black-eyes Susan (*Rudbeckia hirta*)	Prechilling at 5 °C for 6 days	(Aghilian *et al.*, 2014a)
15	Tobacco Sage (*Salvia dorrii*)	Cold stratification at 3-5 °C	(Aghilian *et al.*, 2014a)
16	Common Sage (*Salvia officinalis*)	Pre chilling at 5 °C for 6 days	(Aghilian et al., 2014a).
17	Ginseng (*Panaxginseng*)	cold stratification	(Aghilian et al., 2014a)
18	Echinacea (*Echinacea purpurea*)	Stratification and Scarification	(Aghilian et al., 2014a)
19	St. John's Wort (*Hypericumperforatum*)	Moist stratification at 4 °C	(Aghilian et al., 2014a).
20	Chamomile (*Matricari chamomilla*)	Stratification followed by pre-soaking for 4 h	(Aghilian *et al.*, 2014a)
21	Feverfew (*Tanacetum parthenium*)	Warm stratification	(Brown *et al.*, 1996)
22	Peppermint (*Mentha piperita*)	Stratification and prechilling treatment	(Cooper *et al.*, 2023)

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23	AloeVera (*Aloebarbadensis*)	Chemical soaking in KNO_3 for 6h	(Kulkarni *et al.*, 2014)
24	Arnica (*Arnica montana*)	Warm stratification	(Balabanova *et al.*, 2013)
25	Catnip (*Nepeta cataria*)	Cold stratification at 5 °C for 5 days.	(Aćimović *et al.*, 2021)
26	Perwinkle (*Vinca rosea*)	Chemical soaking followed by warm stratification	(Mohammadzadeh -Shahir *et al.*, 2019)

MORPHOLOGICAL DORMANCY

It is characterized by seeds possessing underdeveloped embryos in terms of size, yet these embryos are differentiated into structures such as cotyledons and hypocotyl-radical. Seeds exhibiting this kind of dormancy are not physiologically dormant but rather require time for growth and germination. For instance, celery (*Apium graveolens*) exemplifies this dormancy (Jaganathan, 2020). The assertion that underdeveloped embryos represent the ancestral state of dormancy in seed plants suggests that this form of dormancy predates other forms such as physiological dormancy, morphophysiological dormancy and physical dormancy (Yaqoob & Nawchoo, 2017)–. Seeds exhibiting morphological dormancy have evolved strategies to persist in the soil seed bank until all the requirements are fit for germination and seedling establishment. This dormancy mechanism allows plants to optimize their reproductive success by timing germination with favorable environmental conditions, thereby increasing the likelihood of seedling survival and establishment. Stratification ~~methods also~~methods also helps to overcome this type of dormancy.

MORPHOPHYSIOLOGICAL DORMANCY

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It has a lot in common with morphological dormancy because it involves seeds that have immature embryos. Nonetheless, seeds exhibiting this also have a physiological aspect to their dormancy, which calls for a dormancy-breaking intervention such as the application of gibberellic acid or warm and/or cold stratification. There are eight recognized levels. *Trollius* (Ranunculaceae) and *Fraxinus excelsior* (Oleaceae) species are two examples (Balabanova et al., 2013) .

COMBINATIONAL DORMANCY

It is characterized by seeds possessing water-impermeable coats, as seen in physical dormancy, along with physiological embryo dormancy. Examples of species displaying dormancy include *Geranium* and *Trifolium* (Baskin & Baskin, 2004). Medicinal plants often have specific requirements for germination, which may include exposure to particular temperature regimes, light conditions, or even chemical cues. Combinational dormancy in medicinal plant seeds may arise as an adaptive strategy to ensure that germination occurs under favorable conditions for seedling establishment and survival (Cho *et al*., 2020) . For example, seeds may exhibit both physiological dormancy, where internal physiological processes inhibit germination until certain conditions are met, as well as physical dormancy, where impermeable seed coats prevent water uptake and germination until they are sufficiently scarified or broken down (Table 3).

Table 3. List of ~~Combinational~~ combinational dormancy and their breaking methods

SN	MEDICINAL PLANT	TYPE OF DORMANCY	BREAKING METHODS	REFERENCE
1	Anise (*Pimpinella anissum*)	Combinational dormancy	Scarification by sand and stratification at 20 °C	(Aghilian *et al.*, 2014a)
2	Rue (*Ruta graveolens*)	Combinational dormancy	Stratification and scarification in mechanical scarifier for 12 min	(Aghilian *et al*., 2014a)
3	Milk Thistle (*Silybum marianum*)	Combinational dormancy	Scarification and stratification at 3 °C for 48 days.	(Pourreza & Bahrani, 2012)

CONNECTING THE DOTS - DORMANCY AND QUIESCENCE IN BIOLOGICAL ADAPTATION

A condition of dormancy during which seeds are not actively growing or ~~germination~~ germinating, but are still viable, is known as the quiescence period in seeds. At this time, seeds are essentially in a "resting" state, with little metabolic activity and little cellular activity. Quiescence is the character of seeds that enables them to survive for long periods of time in unfavourable environments until they are ready for germination. Quiescence is distinct from other types of dormancy in seeds, such as embryo dormancy or hibernation imposed by the seed coat, in that it is not always brought on by environmental cues like light or temperature. Rather, it is a natural characteristic of some seeds and is frequently genetically programmable. Seeds can emerge from their quiescent state and resume active growth and germination when the proper circumstances are met (Velappan *et al.*, 2017).

PLANT HORMONES AND THEIR MULTIFACETED ROLES IN SEED DORMANCY REGULATION

The opposing forces ABA and ~~Gibberellic~~ gibberellic acid (GA) control seed dormancy. While mutants lacking GA biosynthesis or signaling have more dormancy, mutants lacking ABA biosynthesis or signaling often have less dormancy in seeds. This suggests that whilst GA promotes germination, ABA serves to induce dormancy. Gibberellic acid ~~GA~~ is hypothesized to have two effects on dormancy. It does this by first causing the expression of enzymes that weaken the endosperm and make room for the embryo to grow. Secondly, it directly increases the embryo's capacity for growth. According to experiments, ABA may control GA responses in the endosperm, where GA operates predominantly to stimulate the

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break of dormancy (Obroucheva, 2012). There is strong evidence that the ABA and GA signaling pathways interact in the embryo. The degree of dormancy in an embryo may be influenced by the balance of ABA and GA sensitivity, as mutations that damage one pathway might partially attenuate abnormalities in the other (Brady & McCourt, 2003). This partial suppression, however, suggests that distinct ABA and GA pathways exist in parallel. Thus, ABA and GA have opposing and complementary effects on endosperm and embryo in the intricate hormonal regulation of seed dormancy (Shu *et al*., 2015). Genetic regulatory networks of great complexity are involved in the crosstalk between ABA and GA_3, While DELLA (aspartic acid–glutamic acid–leucine–leucine–alanine) proteins are important in GA signaling pathways, transcription factors like ABI3 (ABA-INSENSITIVE 3) and ABI5 are crucial in mediating the effects of ABA on dormancy. In the promoter regions of target genes, interactions between ABA-responsive elements (ABREs) and GA-responsive elements (GAREs) coordinate the transcriptional responses to ABA and GA_3 during dormancy regulation. Known for its role in senescence in plants and fruit ripening, ethylene has been found to be essential in a variety of physiological processes, such as the escape of dormancy in seeds, the development of root hairs, and reactions to viruses and wounds, among other stimuli (Finkelstein, 2004). Many plant species, including peanuts, apples, redroot pigweed, cocklebur, lamb's quarters, *Amaranthus retroflexus*, and sunflowers, have been discovered to break their dormancy when exposed to ethylene. Notably, research with the Arabidopsis ga-1 mutant has demonstrated that, in light settings, ethylene gas can cure germination problems; however, it will cause the seedlings to exhibit the distinctive triple response. This implies that certain ethylene signaling components are involved in the process of germination. The exact genetic processes by which ethylene affects seed germination, however, are still unknown (Jhanji *et al.*, 2024).

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It may become clearer whether ethylene promotes germination by modifying ABA or GA levels or sensitivity, or whether it works through different signaling components, by investigating its interactions with important regulators of ABA and GA production and signaling pathways in more detail. There might also be linkage with other signaling channels that are not yet known to exist according to traditional ethylene response mutant screening (Seo *et al*., 2011).

IMPACT OF DIFFERENT DORMANCY BREAKING TREATMENTS IN MEDICINAL PLANT SEEDS

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Seed germination is a multifaceted physiological process shaped by environmental cues like water availability, light exposure, and other variables. The challenge of limited seed germination significantly hampers the cultivation of threatened medicinal plants, especially in harsh cold desert environments. Natural inhibitors derived from compounds like benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic acid, and abscisic acid (ABA) play pivotal roles in regulating germination (Shu et al., 2015). Certain temperate species experience a warm phase that is followed by a cold one, which breaks their dormancy division into groups. The most common correlation between this reaction and morpho-physiological dormancy is that seeds that exhibit this response have embryos that are not fully formed (Atia *et al.*, 2006).

To expedite this method further, it can be enhanced through the integration of additional treatments such as chemical applications or mechanical seed coat removal (Eberle *et al.*, 2014). Various researchers have explored the impact of exogenous growth regulators on seed germination. For instance, Gibberellins have been found to eliminate the chilling requirements of peach and apple seeds, thereby enhancing their germination rates (Eberle *et al.*, 2014). Recent investigations have indicated that cold stratification directly influences the production of gibberellins (GAs) in seeds of *Arabidopsis thaliana* (Masferrer *et al.*, 2002) externally applied GA has been shown to overcome seed dormancy in multiple species (Hassan & Mathesius, 2012) and to stimulate germination in species that typically necessitate cold stratification, light exposure, or after-ripening (Kandari *et al.*, 2008). ~~standard~~ Standard procedures for enhancing the germination of dormant seeds involve pre-chilling, scarification, and treatments with gibberellic acid (GA_3) or nitric acid (KNO_3).

Various methods, including scarification, using plant growth regulators (PGRs) as a pretreatment ~~(PGRs)~~, and temperature shocks, are employed to break dormancy, depending on the plant species and dormancy type (Copeland & McDonald, 2012; Hidayati et al., 2012). Seed germination is influenced by both intrinsic and extrinsic factors, with certain PGRs playing a crucial role. Abscisic acid (ABA) is involved in germination inhibition, while gibberellins (GAs) participate in terminating seed dormancy (Dewir et al., 2011; Iwasaki et al., 2022). ~~PGRs~~ Plant growth regulaters commonly enhance seed germination capacity, increase biomass yield, and confer resistance to diseases and adverse growth conditions (Papadopoulos *et al.*, 2006). ~~GAs~~Gibberellin acids, synthesized by seeds, are believed to hydrolyze storage nutrients and directly impact embryo growth~~.~~. External application of PGRs, including auxins like Indole-3-acetic acid (IAA), Indole-3-Butyric Acid (IBA) and 1-Naphthaleneacetic acid (NAA)~~.~~, ~~has~~ have been found to break seed dormancy and enhance seedling establishment in various aromatic and medicinal plants (Al-Hawezy, 2013; Kandari *et al.*, 2008; Sivakumar *et al.*, 2006).

Auxins, particularly IAA, have been shown to effectively improve seed germination in *Allium victorialis* (Jeong *et al.*, 2015). IAA and IBA were reported to have a promotive effect on seed germination. NAA increased seed germination in Mentha arvensis by 32.3% (Niu *et al.*, 2019). In *Asparagus sprengeri*, different auxins (IAA, IBA, and NAA) improved seed germination by 10% to 20% compared to the control, while GA_3 was the most effective, increasing seed germination by 47% (Dhoran and Gudadhe, 2012).

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The study on *Ochradenus arabicus* seeds revealed GA_3 as the most effective in overcoming dormancy, resulting in 80-89% germination when treated with 100µM GA_3 and stored for up to 12 months. Increasing GA_3 concentration showed an inhibitory effect on seed germination, and seeds treated with different chemicals exhibited asynchronous germination. These findings suggest that GA_3 is the most effective in promoting seed germination in *O. arabicus*, emphasizing the necessity for hormone application to break dormancy, as documented by Yücel & Yilmaz, (2009). The dormant nature of *O. arabicus* seeds may be attributed to inhibitors, a hard seed coat, low internal hormones, or underdeveloped embryos, aligning with reports on the application of gibberellic acid to alleviate innate and environment-induced dormancy (Yarnia & Tabrizi, 2012) .The seeds of glory lily soaked in boiled water (100 °C) for 40 minutes recorded the highest germination of 62 per cent accompanied with faster rate of germination. This is because the seed coat gets weakened by the hot water and eventually the lignins and pectins present in the epidermal layer of seed gets dissolved (Venudevan *et al.,* 2010). Hence there are numerous ways to break down the dormancy present in seeds according to its nature.

SCARIFICATION

Scarification techniques have been evolved ~~during~~ over time and has become practically feasible for all the crops. Some of the notable methods of scarification include heat, freeze-thaw, mechanical and chemical. Heat scarification uses high temperature to break the hard seed coat whereas freeze thaw breaks the seed coat by alternating temperatures ~~to~~ between high and low. Mechanical scarification breaks the seed coat or makes an injury over it in order to accelerate the seed's rate of imbibition (Khanduri & Negi, 2010) .

HEAT SCARIFICATION

Heat scarification is way more a much easier method due to its easy application. Two main devices that are being applied to scarify are oven and hot water bath. Its efficacy depends on various factors like treatment, time , temperature and seed used. It differs from seed to seed like some may break at low temperature and some may need to be subjected to very high temperatures (Górnik *et al*., 2023) .

The primary purpose of heat scarification is to break seed dormancy. For some species, this dormancy is due to a hard seed coat that inhibits water absorption. Heat scarification helps to overcome this barrier, allowing water to penetrate the seed coat and initiate germination. The temperature and duration of heat exposure vary based on the species of seed being treated. Generally, temperatures range from 100 °C to 120 °C (212°F to 248°F), and exposure times can vary from a few minutes to several hours. It's crucial to research the specific necessities for the seeds you are treating, as excessive heat can damage or kill the seeds (Debouza *et al*., 2024) .

DRY HEAT

Seeds can be exposed to dry heat using methods such as placing them in an oven, using hot sand, or applying heat from a heat gun. Dry heat methods are suitable for seeds that are tolerant of low moisture conditions.

MOIST HEAT

Seeds In this method, seeds are subjected to high temperatures in a moist environment, such as steam or hot water. Moist heat scarification is often preferred for seeds that require

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higher moisture levels for germination. When using heat scarification methods, it's essential to take proper safety precautions to avoid injury or damage to the seeds. This includes using heat-resistant containers or equipment, wearing protective gear when handling hot materials, and closely monitoring the temperature to prevent overheating (Priyadharshini & Lekha, 2021).

After heat scarification, seeds should be cooled and then planted promptly to take advantage of the weakened seed coat and initiate germination. It's important to provide optimal growing conditions, including proper moisture, light and temperature, to support seedling development. Heat scarification can be highly effective for breaking dormancy in certain species of seeds, particularly with hard seed coats or seeds from arid environments (Priyadharshini & Lekha, 2021). However, not all seeds require scarification, so it's essential to research the specific germination requirements of the seeds ~~we~~ one is~~are~~ working with. Overall, heat scarification is a valuable technique for promoting germination in seeds that have dormancy mechanisms related to hard seed coats. When done correctly, it can significantly improve germination rates and support successful seedling establishment. Scarification duration differs from crop to crop.

Table 4. Scarification ~~Treatments~~ treatments for ~~Medicinal~~ medicinal plants

SN	CROP	TREATMENT	EFFECT	REFERENCES
1	Saskoon berry seeds (*Amelanchier alnifolia*)	Scarification at 110℃ with heat	Radicle protrusion was earlier	(Górnik et al., 2023).
2	Burclover (*Medicago polymorpha*)	Sandpaper scarification for 10-40 min	Breakage of hard seed coat	(Alane et al., 2016).
3	Barrel Medic (*Medicago truncatula*)	20 min sandpaper scarification followed by mechanical scarifier	Imbibition of water takes place ~~immedietly~~ immediately after soaking	(Nawrot-Chorabik et al., 2021)
4	Arrow leaf (*Myriopteris intertexta*)	Mechanical scarifier for 15 min	Breakage of hard seedcoat	(Choi et al., 2016)
5	Loofah (*Luffa aegyptiaca*)	Mechanical scarifier at 100 rpm for ~~I1~~ I1 min	Increases seed germination	(Chokchaichamna~~n~~kk~~k~~it et al., 2009)

Commented [A29]: Because other people may be interested in your work; not only you.

Commented [A30]: Surely it works for all seeds?

Commented [A31]: For how long?

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FREEZE-THAW SCARIFICATION

The hidden theory behind this method is to make tiny scars on the seed coat and to make it very light so that germination takes place. This scar making method depends on the seed size, shape, water content, intensity, duration etc. In this freeze thaw technology freezer, carbon dioxide, dry ice, snow, liquid air, acetone, liquid nitrogen are combined and used (Farida *et al*., 2020)~~.~~. It has to be noted that temperature below-80℃ or lower may be more effective than the higher one. Cryo scarification, a form of intentional skin scarring, utilizes extreme temperatures to achieve its desired effect. The process involves tightly binding or clamping the skin before subjecting it to freezing temperatures using substances like dry ice or liquid nitrogen. This freezing causes superficial skin damage, leading to blistering and shedding as the skin thaws, ultimately resulting in scar formation. Repeated freezing and thawing deepen the scars, with more extreme temperature changes producing more dramatic effects. Proper aftercare, such as maintaining cleanliness and moisture, is crucial for minimizing scarring and promoting skin healing. Some medicinal plants exposed to freeze thaw are listed below in ~~table~~ Table 5.

Table 5. Freeze ~~Thaw~~ thaw ~~Treatment~~ treatment in ~~Medicinal~~ medicinal plants

SN	NAME OF MEDICINAL PLANT	TREATMENT	REFERENCES
1	Alfalfa (*Medicago sativa*)	Exposing the seeds in -15 for 36 h so that the seed coat becomes brittle	(Farida *et al*., 2020)
2	Ashwagandha (*Withania somnifera*)	Exposing the seeds at -22 ℃ for 180 days followed by placing the seeds in dry ice at -20 ℃.	(Kimura & Islam, 2012)

Commented [A34]: As opposed to dark? I don't understand what you mean with making it light. Or do you mean that the scars weaken the seed coat?

Commented [A35]: Which one? Or how high a termperature?

Commented [A36]: So, are you going to wash and moisturize all the seeds you have scarified?? This does not make sense. I cannot see why anyone would want to use cryo on seeds… it has been developed for treating scars ons human skin. Also, you do not have one single reference for this method. Are you sure it is used to scarify SEEDS?

Commented [A37]: Always refer to Figures and Tables; not figures and tables.

Commented [A38]: Surely true for all plants?

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3	Pepper (*Piper nigrum*)	Placing the seeds in liquid nitrogen for 10 min and soaking the seeds in hot water bath of 100℃ for 10 min.	(Dosmann & Iles, 1997)
4	Brahmi (*Bacopa monnieri*)	Placing of seeds in liquid nitrogen for 180 days.	(Aouadj *et al*., 2022)

PRE-CHILLING TREATMENT

The pre-chilling treatment proved an efficient in overcoming hibernation for *Calendula officinalis, Saponaria officinalis, Echinacea purpurea, Malva silvestrys, Melissa officinalis*, *Plantago psyllium*, and *Rudbeckia hirta*, resulting in increased germination among the 15 species subjected to pre-chilling. The most prime development was noticed in *Plantago psyllium*, with germination increasing from 40 to 99%, and in *Saponaria officinalis*, from 0 to 30% (Aghilian *et al.*, 2014a). Stratification improved germination in five out of six commercial *E. purpurea* seed lots, with the most significant improvement occurring at 10 °C for 10 days. Highest germination of *Echinacea angustifolia* in response to a combination of 250 ppm GA_3 and 4 weeks of pre-chilling was reported by ~~(~~Farhoudi *et al.*, (2021). Probert *et al*. (2009) also found that pre-chilling treatment was effective in enhancing *Saponaria officinalis* germination. In contrast to the present study, ~~An~~ an increase in the germination of *Ferula gummosa* with pre-chilling at 5 °C was noticed by ~~(~~Nadjafi *et al.*, (2006). Al-Hawezy~~,~~ (2013), observed the highest germination of *Dorema ammoniacum* in 30 days at 3 - 4 °C. It has been investigated that pre-chilling is effective in breaking dormancy in *Salvia sp*. The pre-chilling was not effective in breaking dormancy for *Calendula officinalis* ~~has been reported by~~ (Farhoudi *et al.*, 2021). In the case of *Ferula gummosa* and *Dorema ammoniacum*, seeds did not germinate, and pre-chilling had no effect on breaking their dormancy. Nevertheless, both the application of GA3 and pre-chilling at 5 °C stimulated both the rate and final germination of *Ferula gummosa* (Nadjafi *et al.*, 2006). Physiological dormancy in some species can be broken by relatively short periods

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Commented [A41]: Which present study? Since yours is a review of other studies?

Commented [A42]: Reference?

of cold stratification. Thus, species for which pre-chilling was effective in breaking dormancy might possess physiological dormancy. A range of the prechilling treatments for medicinal plants are ~~en~~listed below in ~~table~~ Table 6.

Table 6. ~~list of~~ Medicinal herbs and their ~~p~~Pre-chilling ~~t~~Treatments

SN	MEDICINAL PLANT	PRECHILLING TREATMENT	EFFECT	REFERENCES
1	Corn Poppy (*Papaver rhoeash*)	Wet pre-chilling	Improved germination rate	(Golmohammadzadeh *et al.*, 2015)
2	Long-headed Poppy (*Papaver dubium*)	Wet chilling	Increased seed germination rate from 46% to 90%	(Balouchi & Sanavy, 2006)
3	Alfalfa (*Medicago radiata*)	Pre-chilling followed by soaking of seeds in GA3 at 250ppm for 1 week	Seed germination was easy as the hard seed coat gets softened	(Gosling *et al.*, 2003)
4	California Burclover (*Medicago polymorpha*)	Pre-chilling at 20 °C for a week	Seedcoat was permeable	(Sharifi *et al.*, 2017)
5	Yellow Sweet Clover (*Melilotus radiata*)	Pre-chilling at 4 ℃ followed by soaking in GA3 at 750 ppm	Physical dormancy was broken	(Rahnama-Ghahfarokhi & Tavakkol-Afshari, 2007).
6	Yellow Sweet Clover (*Melilotus rigidula*)	Pre-chilling at 4 ℃	Maturity of embryo was quick	(Balouchi & Sanavy, 2006)
7	Douglas Fir (*Psedostuga menziesii*)	Pre-chilling at 15 ℃ for 128 weeks	Protrusion of embryo	(Ertle, 2020)
8	Asafoetida (*Ferula assafoetida*)	Pre-chilling at 9 ℃ for 90 days	Increased germination percentage	(Keshtkar *et al.*, 2009)

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Commented [A44]: Is this the same as wet pre-chilling?

Commented [A45]: Alfalfa is *Medicago sativa*. *Medicago radiata* is related, but known as one of many medics.

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Commented [A46]: I see you use two difference scientific names for 'yellow sweet clover", but the internet provides me with yet another scientific name:
YELLOW SWEET CLOVER (*Melilotus officinalis*)
I cannot find any reference on the internet to *Melilotus radiata* OR *Melilotus rigidula* **Please double-check.**

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9	Galbanum (*Ferula gummosa*)	Pre-chilling at 5 ℃ and soaking of seeds ij GA3 at 400 ppm	Sprouting of seeds	(Gastmann *et al.*, 2023)
10	Colocynth (*Citrulus colocynthis*)	Pre-chilling at 4 ℃ for 50 days	Dormancy was broken and germination occurred at earlier stage.	(Kahrom *et al.*, 2022)

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GIBBERELLIC ACID (GA_3)

The significant improvement in sprouting of seeds with GA_3 treatment in *Plantago psyllium*, *Rudbeckia hirta* and *Satureja hortensis* compared to untreated seeds were studied~~.~~, indistinguishable results were obtained in *Opuntia tomentosa* (Olvera-Carrillo *et al.*, 2003). Plant growth regulators like GA_3 and KNO_3 seem to play a role in breaking dormancy by establishing hormonal balance and reducing germination inhibitor substances. The results were compatible with those reported by ~~(~~Alp *et al.*, (2008) ~~observation of an~~ who observed an enhanced germination rate in *Cuminum cyminum* with GA_3 application. ~~(~~Petrić et al., (2013) also reported increased germination of *Hyssopus officinalis* seeds with GA_3 pre-treatment. Loquat (*Eriobotrya japonica L.)* being a prime fruit crop that has potential of curative properties is mainly cultivated through seeds. The exogenous growth regulators can modify the digestibility of seed coat and if treated with GA_3 250 mg.L^{-1} with a soaking duration ~~is~~ of 36 h (Al-Hawezy, 2013).

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POTASSIUM NITRATE (KNO_3)

One of the major issues in cultivating medicinal herbs in a commercial way is that they readily germinate in natural or native environment but when they are ~~mandatory to~~ tested for germination under laboratory conditions they ~~get~~ fail to germinate as readily as in the wild.

~~ed~~. Dormancy is not a single phenomenon and it contributes together with various aspects (Finkelstein *et al.*, 2008). KNO_3 was found to be effective in breaking seed dormancy for *Calendula officinalis, Cynara scolymus, Ocimum basilicum, Physalis alkekengi,* and *Satureja hortensis* compared to their germination in H_2O. Similar instances were noted in studies on ~~*Calendula officinalis* and~~ *Echinacea purpurea* (Farhoudi et al., 2021). As mentioned earlier, KNO_3, acting as a plant growth regulator, plays a role in breaking dormancy by establishing hormonal balance and reducing germination inhibitor substances. In an experiment, g~~G~~albanum seeds ~~when~~ where stratified with perlite medium and then subjected to various concentrations of KNO_3 (0, 0.1, 0.2 and 0.3% (v/v)). ~~, after~~ After 7 weeks of stratification, the germination percentile of the seeds soaked in 0.3 percent KNO_3 ~~the germination percentile~~ sho~~o~~t up to 86 % (Sharifi *et al*., 2017).

Commented [A49]: You have already listed *Calendula officinalis*

STRATIFICATION OF SEEDS

Exposure to cold temperatures during stratification mimics the natural winter conditions that many seeds would experience in their native habitats. This cold treatment is particularly effective for seeds of plants adapted to temperate climates (Bratcher *et al.*, 1993). The cold temperatures initiate physiological changes within the seed that break dormancy and prepare it for germination when conditions become favorable. In moist stratification, seeds soak up moisture from their environment. This hydration softens the outer covering and triggers biochemical processes within the seed (Milberg & Andersson, 1998). The cold and sometimes moist conditions of stratification can soften the seed coat, which is often impermeable to water and gases. Softening of the seed coat allows for enhanced water uptake and gas exchange, facilitating metabolic activity within the seed.

Table 7. Stratification ~~Treatments~~ treatments in ~~Various~~ various ~~Medicinal~~ medicinal crops

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SN	MEDICINAL PLANT	TREATMENT	REFERENCES

1	Fennel *Foeniculum vulgare*	Exposing the seeds at 10°C for about weeks	(Hashemirad *et al*., 2023)
2	Echinacea *Echinacea sp*	Exposing the seeds at 4°C for 10 days	(Romero *et al., 2005)*
3	Kalajira *Bunium persium*	Moist stratification at 4 °C for 5 days	(Sharma & Sharma, 2010)
4	Loddon lilly *Leucojum aestivum*	Warm stratification at 30 °C for 6 weeks	(Çiçek *et al., 2007)*

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SECONDARY METABOLITES (SM) - METABOLIC MAESTROS OF DORMANCY AND LONGEVITY

The duration of dormancy is critical for the secondary metabolites. When seeds remain dormant, they protect as a defensive mechanism against a distinct of biotic and abiotic stressors. Certain secondary metabolites, ~~like~~ such as phenolic compounds, have antioxidant properties that shield them from oxidative damage. As a result, they provide protection throughout the dormant phase, raising the likelihood of successful germination in the event that favorable conditions continue.

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SMs found in medicinal plants are essential components that underpin their clinical curative effects and serve as crucial indicators for evaluating the quality of medicinal materials. The synthesis and accumulation of SMs are intricate processes influenced by both internal developmental genetic circuits (such as regulated genes and enzymes) and external environmental factors (including light, temperature, water, and salinity). While existing literature extensively explores the impact of environmental factors on SM synthesis and accumulation, there is a gap that requires a systematic classification and summary of the effects of developmental growth and genetic factors on SMs. The biosynthesis of SMs initiates from basic pathways such as glycolysis or shikimic acid pathways, with subsequent diversification depending on cell type, developmental stage, and environmental cues (Patra *et al.*, 2013). Distinct cells, tissues and organs of medicinal plants may exhibit different medicinal properties at various developmental stages due to the influence of developmental factors on cellular structures involved in SM biosynthesis and storage (Brown *et al.*, 1996). Plant growth and development, influenced or impeded by different environmental conditions, significantly impact the accumulation of secondary metabolites (Zhang *et al.*, 2010). Ashwagandha, being an important medicinal plant of Indian origin, has numerous secondary metabolites. ~~in which~~ In an experimental study (Sanchita *et al.*, 2015)~~Withanaloid~~ withanaloid ~~increases~~ increased during saline stress. ~~is evident in experimental study and~~ It is possible that all the secondary metabolites behave in the same way ~~to express their origin~~ during external stress conditions. ~~(Sanchita *et al.*, 2015)~~. Environmental variations strongly affect SM pathways and their regulation, as the expression of genes involved in SM pathways undergoes alterations in response to different stresses (Borges *et al.*, 2017). Secondary metabolites have a ~~heavy~~ strong interaction with external environment with plants and are initially produced by primary metabolites in higher plants (Ashraf *et al.*, 2018)

Commented [A52]: Because I doubt that this author had tested all 100 000!!

Commented [A53]: Not quite certain what you mean with "express their origin"? This whole paragraph is difficult to read. I have made changes, but it is possible that I didn't get the gist right. Please rephrase, but be clear.

Commented [A54]: Interaction is expressed numerically, thus strong is the better word.

Commented [A55]: Surely the SMs are IN the plants? Or do you mean the surrounding plants? Not clear.

The plant kingdom boasts approximately 100,000 secondary metabolites, classified into three major groups based on bio synthetic pathways: nitrogen-containing compounds (cyanogenic glycosides, alkaloids, and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids), and terpenes (isoprenoids) (Fang *et al.*, 2012). Despite progress in SM biosynthesis and accumulation research, reports on how developmental and environmental factors stimulate the synthesis and accumulation of secondary metabolites in medicinal plants are limited. The stimulation of metabolites biosynthesis in medicinal plants by controlling and optimizing external and internal factors holds promise for advancing bio technologies aimed at high-quality drug production (Perchuk *et al.*, 2020).

Secondary metabolite synthesis, accumulation, and distribution patterns in medicinal plants categorize medicinal parts into four main types: roots and stems, leaves, flowers, and fruits and seeds. The complexity and diversity of these metabolites in different parts of medicinal plants

~~end up~~all contribute to the synthesis of different SMs through specific regulatory pathways and transport routes in certain organs, tissues, and cells (Li *et al.*, 2020). Thus, SM biosynthesis and accumulation exhibit organ or tissue specificity.

Fruits and seeds of medicinal materials in many plants, are influenced by their developmental stages, impacting the content and composition of components. For instance, in ~~Citrus~~ citrus fruits, volatile oil content, the main active ingredient, is highest when the fruit is light yellow, serving as a morphological index for harvesting (Li *et al.*, 2019). Essential oil yields in *Citrus medica* L. var. *sarcodactylis* significantly increase during maturation, and the content of specific compounds varies during maturation stages (Wu *et al*., 2016). Similarly, capsules of *Papaver somniferum L*. reach the maximum morphine content at maturity (Verma & Shukla, 2015). Therefore, the developmental stages of plants significantly affect the content and composition of SMs. The synthesis and accumulation of SMs are also closely associated with the developmental stage of medicinal plant seeds. For example, in coffee, quinic acid content remains relatively stable, but dicoffee quinic acid content decreases noticeably with seed development. The content of quinic acid, a precursor substance for chlorogenic acid synthesis, is high in the early developmental stage of seeds and decreases significantly later on, related to the expression characteristics of the HQT gene, a key enzyme regulating phenolic acid biosynthesis (Awada *et al*., 2023).

CONCLUSION

Since prehistoric times therapeutic plants have ~~many~~ been used for many distinct purposes. They synthesize countless bioactive compounds which are unique to use. Protection and ~~Defense~~ defense activity is one of the significant acts of medicinal herbs. Seed market is expected to be on its peak and hyperactive with hybrid varieties developed indigenously for domestic markets and commercial farmers. Vegetable seed and diversified crops like ~~Medicinal~~

Commented [A56]: Not sure what you mean here. Maybe: .. expected to be at its peak and filled with hybrid varieties. Since “hyperactive” is usually used for people who cannot sit still.

medicinal and ~~Aromatic~~ aromatic plants will ever have enormous scope ~~to win~~ and will play an important role in economy. This review critically points out the studies carried out over time. So, studies on these medicinal plant remain~~s~~ to be a growing part for further research. Dormancy pertaining to the seeds must be considered as in boon and bane way according to the various external factors. Sometimes ~~this bane~~ dormancy helps in seeds' preservation and in other cases it is considered as serious ~~issue~~ disadvantage for germination. Since the presence of seeds itself is big drawback considering these issues can lead to a big boon in pharmacological industry for availing drugs at an earlier and more cost efficient way.

Commented [A57]: Who is winning what?

Commented [A58]: This is not scientific language. I suggest: Dormancy in seeds has advantages and disadvantages, depending on various external factors.

Commented [A59]: I do not understand. If seeds are a drawback, how would many plants reproduce? Maybe: Considering and being able to manipulate the issue of breaking dormancy in seeds can lead to a more stable supply of raw material for the pharmacological industry. But please, boon and bane is NOT scientific language.

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