

Clonal Field Evaluation of *Morus Sinensis* (MR2) Mini Clones Impacted by Different Transplanting Days on Field Survivability and Growth Attributes and Analyzing Its Therapeutic Potential

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ABSTRACT

Morus sinensis (MR2) is an important mulberry species cultivated for its high nutritional value and adaptability to various environmental conditions. This study evaluates the apical cuttings of *Morus sinensis* (MR2) mini clones under field conditions to determine their growth performance and its therapeutic potential. Mini clones 60DAP-AC (MR2) registered high survival percentage of 88.21 per cent under field conditions. In MR2 variety, mini clones 60DAP-AC performed well with respect to attributes like plant height (112.12cm) and inter-nodal distance (5.21cm) and leaf area (129.68cm²). Validating field survival and growth characteristics of mini clones under primary field conditions with regard to various transplantation days was the study's primary goal. Additionally, phytochemical analysis of *Morus sinensis* (MR2) leaves demonstrated the presence of bioactive compounds such as flavonoids, alkaloids, polyphenols and terpenoids which are known for their antioxidants, anti-inflammatory and antidiabetic properties. These findings suggest that optimizing transplanting days not only enhances field establishment but also contributes to improved biomass production and bioactive compound accumulation. The study underscores the potential of *Morus sinensis* (MR2) in sustainable agriculture, sericulture and herbal medicine. Further research on the pharmacological properties of its bioactive compounds could provide deeper insights into its therapeutic applications thus paving the way for its inclusion in nutraceutical and pharmaceutical formulations.

Key words: clonal test, field survival, growth parameter, mini clones, mulberry, therapeutic potential

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INTRODUCTION

Mulberry MR2 variety (*Morus sinensis*.) is a rapidly growing deciduous woody perennial that is typically pruned repeatedly to form a shrub or dwarf tree (Magadum *et al.*, 2019; Bharathi *et al.*, 2022). Since it is the main source of food for silkworms

(*Bombyx mori* L.), the viability and profitability of the sericulture sector are significantly impacted by the availability of high-quality leaves (Rebai *et al.*, 2017; Janakirama *et al.*, 2020). Leaf yield is significantly impacted by spacing. The plantation system also affects the quality of mulberry leaves.

There is evident competition for air, light, soil moisture and nutrients *etc.* because there is not enough space which results in low yield (Bongale, 1991). Plant growth including plant height, number of branches/plant, shoot length, number of leaves/plant and leaf yield/plant which is directly impacted by spacing (Lim and Choi, 2019; Mahesh *et al.*, 2017).

The therapeutic applications of *Morus* species span several health conditions including metabolic disorders (diabetes & obesity), neurodegenerative diseases (Alzheimer's & Parkinson's), cardiovascular diseases and cancer (Rodrigues *et al.*, 2019; Kadam *et al.*, 2019). Extracts from different parts of the plant such as leaves, fruits, bark and roots exhibit antioxidant, anti-inflammatory, anti-diabetic, hepatoprotective and neuroprotective properties (Kebai *et al.*, 2017; Dhiman *et al.*, 2020). Notably, mulberry leaves and roots contain high concentrations of 1-deoxynojirimycin (DNJ), a potent α -glucosidase inhibitor that plays a crucial role in glucose metabolism regulation (Ramesh *et al.*, 2014). Given the increasing interest in plant-based therapeutics and the ongoing search for natural alternatives to synthetic drugs, the pharmacological properties of *Morus* species need a comprehensive investigation (Janakirama *et al.*, 2020; Abdelrazak *et al.*, 2023).

The fruits of *Morus sinensis* is sorosis type and used for dishes which is extremely perishable. Mulberry fruits have long been utilized for their therapeutic qualities which include blood feeding, renal advantages, treatment of weakness, exhaustion, anemia and early hair graying (Mahesh *et al.*, 2017). Mulberry juice has many anti-aging benefits including enhancing immunity, calming nerves, balancing internal secretions and protecting the liver from harm (Yadav *et al.*, 2015). It also nourishes the blood. Mulberry fruits contain anthocyanins that have anti-oxidant properties which can scavenge free radicals, prevent low-density lipoprotein oxidation and improve blood lipid and atherosclerosis (Duyen *et al.*, 2013). For every 100 grams of edible flesh, fruit has 85.5% moisture, 0.7% protein, 0.4% fat and minerals, 12.2% carbohydrates and 10 mg vitamin C (Singhal *et al.*, 2010).

When compared to stem cuttings, mini clonal technology offers an alternate method for producing huge numbers of plants in a short amount of time and space. It also shows improved rooting potential, speed and quality root system at a lower cost (Parthiban, 1999). Numerous tree species including *Melia dubia*, *Casuarina* sp., *Eucalyptus* sp., *Tectona grandis* and *Dalbergia sissoo* have been successfully cut using the mini cutting technique (Sabarish, 2017; Parthiban *et al.*, 2021). Using the juvenile cuttings as a source of planting material is the primary goal of the mini clonal technique (Bharathi *et al.*, 2022). Apical shoots are used as mini cuttings, which are put in into a rooting medium in a greenhouse that has humidity and temperature controls adjusted appropriately (Seenivasan and Parthiban, 2017; Kiruthika, 2020). This study aims to explore the field survival, growth potential and therapeutic benefits of *Morus* species by highlighting their bioactive compounds, mechanisms of action and potential applications in modern medicine.

MATERIALS AND METHODS

The research was conducted during October 2021 to June 2022 at Department of Sericulture and Clonal complex at Forest college and Research Institute, Tamil Nadu Agricultural University, Mettupalayam. (11°20'N, 76°55'E, 300 meters above mean sea level with mean rainfall of 800mm). The stem and apical cuttings of MR2 commercial varieties of mulberry were raised in clonal complex nursery and plants from treatment performed better in clonal nursery were taken to main field condition.

Rooting hormones and media for mini-clonal propagation of mulberry

Auxin is produced naturally in plants at their growth tips and then transferred to other parts of the plant. A class of phytohormones that stimulate plant growth in a longitudinal direction. Mini cuttings that had been excised were exposed to exogenously produced auxins such as Indole-3 butyric acid, which promote the development of the roots. Boric acid crystal, talc, fungicide and IBA powder are the ingredients of rooting hormone. Among varied rooting hormone concentration *viz.* 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm. Plants treated at 5000 ppm IBA performed well. IBA @ 5000 ppm was therefore the ideal rooting hormone for the MR2 mulberry (Sabarish, 2017). The excised apical cuttings were placed in root trainers after being dipped in the proper rooting hormone at the required concentration. The rooting and shoot development of the cuttings will be greatly influenced by the rooting medium. A 1:1:1 combination of soil, coir pith and FYM was the ideal composition for rooting media (Kiruthika, 2020). The apical cuttings were housed in an inexpensive poly-tunnel construction and planted without causing any harm to the basal cambium section. The temperature inside the polytunnel was kept at 30°C $\pm$ 3°C, and 80–85% relative humidity was the target. There was one weekly irrigation provided.

To study the effect of different transplanting days on survival and growth parameters of MR2 apical cuttings under field conditions

The healthiest and best-performing mini clones at the nursery level were identified for the study. The mini clones (apical cuttings) and stem cuttings of MR2 mulberry varieties were evaluated using varying transplanting days as treatments, namely 50 DAP, 60 DAP, 70 DAP, 80 DAP and 90 DAP. Additionally, 90-day-old mulberry saplings produced using stem cutting method of propagation were used as a check. The main field was leveled and tilled to a fine texture. Wider plant spacing of 10 feet by 10 feet was used to prevent plant interaction and assess the full potential of mini clones in field situations. At the proper planting dates, stem cuttings and mini clones were transplanted. Every five days, there was one irrigation. Sixty days following the initial transplant process, weeding was completed. Using a standard package of practices, four replications and ten plants per replication were used in the Factorial Randomized Block Design (FRBD) trials. At 30, 60 and 90 days interval, observations were made regarding the following physiological, growth and survival parameters: leaf area (cm²/plant), plant height (cm), inter-nodal distance (cm) and field survival (%).

Experimental Analysis

The extraction, isolation and identification of bioactive compounds from *Morus* species were done. Methods such as Gas Chromatography-Mass Spectrometry (GC-MS) and specific procedures for each compound were considered to understand the phytochemical profile of *Morus* extracts. Extracted information was systematically categorized based on therapeutic effects including antioxidant, anti-inflammatory, anti-diabetic, neuroprotective, hepatoprotective and anticancer properties. The findings were critically analysed to determine potential mechanisms of action, efficacy and future research prospects.

Treatments

T1: Variety MR2 transplanting on 50th day (Apical cuttings)
T2: Variety MR2 transplanting on 60th day (Apical cuttings)
T3: Variety MR2 transplanting on 70th day (Apical cuttings)
T4: Variety MR2 transplanting on 80th day (Apical cuttings)
T5: Variety MR2 transplanting on 90th day (Apical cuttings)
T6: Variety MR2 transplanting on 90th day (Stem cuttings) – check

Observations recorded

The observations were recorded on survival, growth and physiological attributes of mulberry

Field survivability

Formula was used to determine the percentage of mini-clones that survived in field conditions on 50 DAT.

Number of plants survived

Field survival per cent of clones = -----
----- × 100

Number of plants
planted

Mulberry growth attributes

Five randomly chosen mulberry plants in main field circumstances were labeled for the purpose of recording growth parameters from each replication.

Plant height

The entire distance between a plant's tallest point and its lowest point at ground level is called plant height. Most of the time, it was described in centimeters.

Inter-nodal distance

The length measured between the nodes with the fourth and fifth leaves from the plant's tallest tip was recorded. Generally, the measured mean value was represented in centimeters.

Leaf area (LA)

Ten mulberry plants make up each replication, of which five were chosen at random. The fifth leaf from the tip of the plant was then collected, and the leaf area was measured using Montgomery's suggested length and breadth method (1911). The mean value was given as cm²/plant.

Leaf Area = l b c

Where, A- leaf area, l – leaf length, b – leaf breadth, c – constant factor (0.676)

Statistical analysis

The treatments that were determined to be significant and the critical differences were computed and analyzed at the five percent probability level during the statistical analysis of the data. In order to critically analyze the data gathered from the previously mentioned trials, Fisher's (1926) Factorial Randomized Block Design (FRBD) was used. The stage-by-stage data was analyzed using the AGRES software tool (version 0.74).

RESULTS AND DISCUSSION

In MR2 variety, at 60 DAP-AC (T2), the field survivability ranged from 84.55 per cent to 92.12 per cent and the mean value was recorded at 88.21 per cent. For 90 DAP-SC (T6), the mean survivability differed from 83.10 per cent to 87.26 per cent and the mean value was 84.81 per cent (Figure 1). From the results of field survivability, treatment T2 (88.21%) and T6 (84.81%) are statistically on par with each other. Higher field survivability was recorded in treatment T5 (90 DAP-AC) followed by T4 (80 DAP-AC) and poor field survival rate (74.80%) was witnessed in T1 (50 DAP-AC) (Figure 1). The field survival of mini clones will be directly impacted by the hardening time. The increase in field survivability was due to well-developed root system in mini clones and also due to increased water and nutrient intake efficiency. Similar findings reported by Parthiban *et al.* (2021). Sudhakar *et al.* (2018) found that increased survival rate noticed in clones compared to stem cuttings.

In MR2, T5 registered maximum plant height of 64.37cm, 89.78cm and 119.10cm on 30, 60 and 90 DAT respectively. This was followed by T4 were 61.72cm (30 DAT), 85.40cm (60 DAT) and 117.68cm (90 DAT) and T1 recorded lowest plant height 46.61cm, 62.83cm and 89.21cm on 30, 60 and 90 DAT respectively (Table 1). From statistical analysis, it was found that for MR2, T2 (112.12cm) and T3 (112.96cm) was on par statistically at 90 DAT. It was observed enhanced vigour in plants generated from apical buds compared to lateral buds. The rate of cell multiplication is faster in growing tips rather than lateral buds. These findings are in confirmation with Sudhakar *et al.* (2018) who noticed increase in plant height of 48.7 per cent in clones compared to traditional propagation method. It was also supported by Sudhakar *et al.* (2020) who recorded significant difference in growth attributes of high yielding mulberry varieties at 45, 60 and 90 days after planting.

In MR2, T5 found to have maximum inter-nodal length of 3.52cm, 4.39cm and 5.44cm on 30, 60 and 90DAT respectively. This was followed by T4 were 3.36cm (30 DAT), 4.30cm (60 DAT) and 5.38cm (90 DAT) and T1 registered lowest inter-nodal length of 2.33cm, 2.69cm and 3.43cm on 30, 60 and 90 DAT respectively (Table 2). The values obtained were subjected to statistical analysis, in MR2 variety at 90 DAT, T3 (5.33cm), T4 (5.38cm) and T5 (5.44cm) was on par statistically. The plant's growth was directly correlated with an increase in inter-nodal length. An inter-nodal distance of 5.2 cm in the V1 variety at 90 DAT was measured, further supporting these observations by Sudhakar *et al.* (2020).

In MR2, T5 found to have maximum leaf area of 118.68cm², 125.81cm² and 136.22cm² on 30, 60 and 90 DAT respectively.

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This was followed by T4 were 116.63 cm² (30 DAT), 124.19cm² (60 DAT) and 132.36cm² (90 DAT) and T1 registered lowest area of 97.68cm², 106.21cm² and 115.86cm² on 30, 60 and 90 DAT respectively (Figure 2). For MR2, all the treatments except T1 (115.86cm²) was on par statistically at 90 DAT. Difference in growth period was due to hardening treatment, variation among the species and physiological characters of MR2 variety have influenced leaf area measurements. These findings were supported by Mithilasri *et al.* (2021).

Morus sinensis contains a variety of bioactive compounds that contribute to its extensive therapeutic applications (Kadam *et al.*, 2019). Among these, 1-Deoxynojirimycin (DNJ) is a key anti-diabetic compound predominantly found in mulberry leaves and bark (0.1–2.0%). DNJ acts as an α -glucosidase inhibitor thus reducing postprandial blood glucose levels and improving insulin sensitivity (Wulandri *et al.*, 2019). Flavonoids such as quercetin (1.5–5.0 mg/g), rutin (2.0–8.0 mg/g) and kaempferol (0.5–3.5 mg/g) are abundant in mulberry leaves and fruits (Nastic *et al.*, 2018). These compounds exhibit potent antioxidant and anti-inflammatory properties by

scavenging free radicals and inhibiting pro-inflammatory cytokines (Yang *et al.*, 2017). Anthocyanins (5.0–25.0 mg/g), primarily found in mulberry fruits, further enhance antioxidant capacity and provide neuroprotective benefits (Espada *et al.*, 2018).

The plant also contains morusin (0.2–2.5 mg/g) and isoflavones (1.0–6.0 mg/g) in its root bark known for their anticancer properties (Abdulrazak *et al.*, 2023). These compounds induce apoptosis in cancer cells and inhibit tumour growth (Chen *et al.*, 2013). Additionally, mulberroside A (0.3–3.0 mg/g) and chlorogenic acid (1.0–4.5 mg/g) found in leaves and root bark contribute to hepatoprotective effects by reducing lipid accumulation and preventing liver fibrosis (Elbaz *et al.*, 2017). Furthermore, phenolic acids (10–40 mg/g) and polysaccharides (5–15%) support immune modulation and metabolic health, while betulinic acid (0.1–1.5 mg/g) exhibits antimicrobial and anti-inflammatory activities (Kostic *et al.*, 2019). These compounds work synergistically to protect against cardiovascular diseases, diabetes and neurodegenerative disorders (Janakirama *et al.*, 2020).

Table 1. Effect of different transplanting days on plant height (cm) of MR2 mini clones at different time intervals

Treatment	30DAT	60DAT	90DAT
50 DAP-AC (T1)	46.61 ^c	62.83 ^d	89.21 ^d
60 DAP-AC (T2)	54.05 ^b	79.9 ^{bc}	112.12 ^b
70 DAP-AC (T3)	57.83 ^{ab}	82.93 ^{bc}	112.96 ^b
80 DAP-AC (T4)	61.72 ^a	85.4 ^{ab}	117.68 ^{ab}
90 DAP-AC (T5)	64.37 ^a	89.78 ^a	119.1 ^a
90 DAP-SC (T6)	57.29 ^{ab}	78.62 ^c	104.34 ^c
SE(d)	3.24	2.63	2.75
CD(0.05)	7.28**	5.86**	6.13**

Note: AC - Apical cuttings; SC - Stem cuttings

**Highly significant, *Significant

Each value is the mean of four replications

Means followed by same alphabets are on par with each other by LSD (P=0.05)

Table 2. Effect of different transplanting days on inter-nodal distance (cm) of MR2 mini clones at different time intervals

Treatment	30DAT	60DAT	90DAT
50 DAP-AC (T1)	2.33 ^c	2.69 ^c	3.43 ^c
60 DAP-AC (T2)	3.21 ^{ab}	4.15 ^a	5.21 ^{ab}
70 DAP-AC (T3)	3.24 ^{ab}	4.23 ^a	5.33 ^a
80 DAP-AC (T4)	3.36 ^a	4.3 ^a	5.38 ^a
90 DAP-AC (T5)	3.52 ^a	4.39 ^a	5.44 ^a
90 DAP-SC (T6)	3.03 ^b	3.75 ^b	4.81 ^b
SE(d)	0.14	0.11	0.22
CD(0.05)	0.32**	0.25**	0.49**

Note: AC - Apical cuttings; SC - Stem cuttings

**Highly significant, *Significant

Each value is the mean of four replications

Means followed by same alphabets are on par with each other by LSD (P=0.05)

Table 3. The approximate composition of key bioactive compounds in *Morus sinensis*

Compound	Approximate Composition (% or mg/g dry weight)	Plant Part	Reference
1-Deoxynojirimycin (DNJ)	0.1–2.0%	Leaves & Bark	Wulandri <i>et al.</i> , 2019
Quercetin	1.5–5.0 mg/g	Leaves & Fruits	Yang <i>et al.</i> , 2017
Rutin	2.0–8.0 mg/g	Leaves & Fruits	Nastic <i>et al.</i> , 2018
Anthocyanins	5.0–25.0 mg/g	Fruits	Espada <i>et al.</i> , 2018
Kaempferol	0.5–3.5 mg/g	Leaves & Bark	Chen <i>et al.</i> , 2021
Morusin	0.2–2.5 mg/g	Root & Bark	Chen <i>et al.</i> , 2013
Mulberroside A	0.3–3.0 mg/g	Leaves, Root & Bark	Elbaz <i>et al.</i> , 2017
Chlorogenic acid	1.0–4.5 mg/g	Leaves & Fruits	Espada <i>et al.</i> , 2022
Phenolic acids	10–40 mg/g	Leaves & Fruits	Kostic <i>et al.</i> , 2019
Polysaccharides	5–15%	Leaves & Fruits	Yang <i>et al.</i> , 2017
Betulinic acid	0.1–1.5 mg/g	Bark & Root	Chen <i>et al.</i> , 2022
Isoflavones	1.0–6.0 mg/g	Root & Bark	Chen <i>et al.</i> , 2022

Note: These values are approximate and may vary based on specific experimental conditions.

Figure 1. Effect of different transplanting days on field survivability (%) of MR2 mini clones

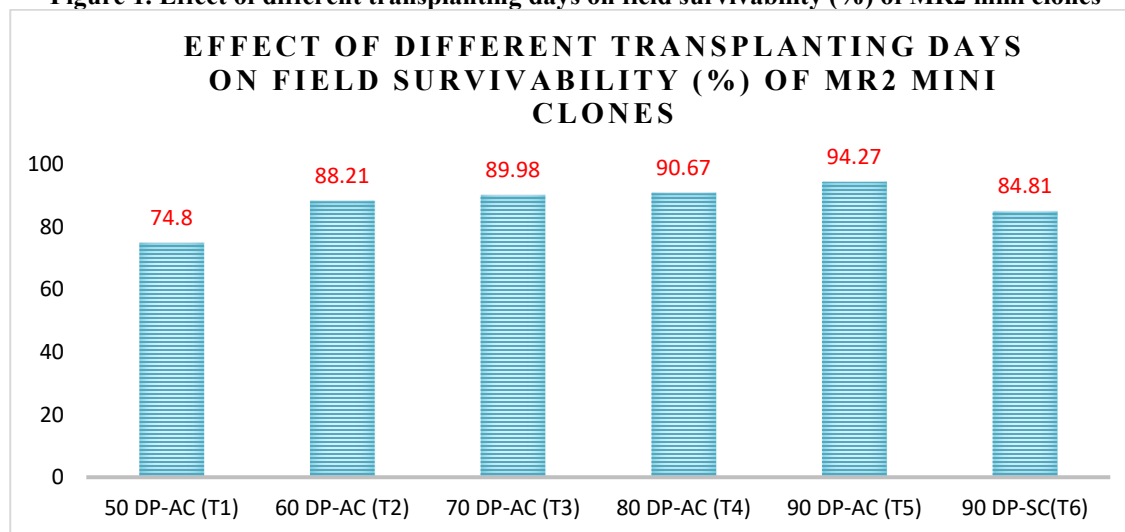
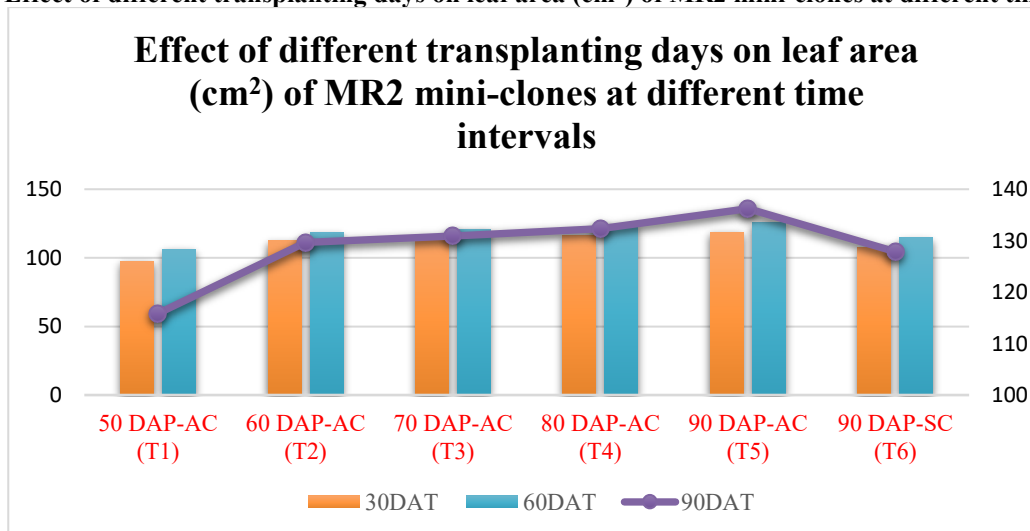


Figure 2. Effect of different transplanting days on leaf area (cm²) of MR2 mini-clones at different time intervals



CONCLUSION

Mini clones 60 DAP-AC (MR2) recorded high survival percentage of 88.21 % compared to stem cuttings. Mini clones 60 DAP-AC (MR2) registered maximum plant height of 112.12 cm. Mini-clones 60 DAP-AC (MR2) showed mean nodal length of 5.21cm. Mini clones 60 DAP-AC (MR2) recorded maximum leaf area of 129.68 cm² respectively when compared to check. Plant generated using stem cuttings registered plant height (104.34 cm), internodal distance (4.81 cm), leaf area (127.88 cm²) and field survival rate (84.81 %). The evaluation of *Morus sinensis* (MR2) mini clones under field conditions and their therapeutic potential highlights the plant's adaptability, agronomic viability and pharmacological significance. The successful cultivation of MR2 mini clones in diverse environmental conditions underscores their potential for large-scale propagation thus ensuring a sustainable supply of bioactive compounds. Additionally, phytochemical analysis confirms the presence of flavonoids, alkaloids and phenolic compounds, which contribute to the plant's antioxidant, anti-inflammatory and antimicrobial properties. The therapeutic potential of MR2 mini clones extends to various biomedical applications including antidiabetic, neuroprotective and cardioprotective effects. These findings suggest that *Morus sinensis* MR2 could serve as a valuable resource for developing natural health supplements and pharmaceutical formulations. However, further clinical research is necessary to validate these bioactivities, optimize extraction methods, ensure efficacy and safety for human applications. In conclusion, *Morus sinensis* (MR2) mini clones demonstrate promising agronomic performance and therapeutic potential thus making them a viable candidate for both commercial cultivation and medicinal applications. Future research should focus on large-scale field trials, clinical studies and mechanistic investigations to fully harness the medicinal benefits of this plant.

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