Significance of Tissue Culture in Medicinal Plant Conservation and enhancement of Secondary metabolites by Homoeopathic intervention: A Narrative review

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Accepted for publication: 2023-12-27
Published: 2024-06-30

ABSTRACT

Aim: The objective of this study is to explore the potential of homoeopathic remedies in enhancing the biosynthesis of secondary metabolites in endangered medicinal plants. Background: Plants serve as a valuable source of medicine. Plant-based medicines are known for their simplicity, efficiency, and a broad range of activity with well-documented preventative or therapeutic effects. Secondary metabolites are active chemical components found in plants that are responsible for their medicinal properties. However, population growth, increased demand, and illicit trafficking is driving the depletion of medicinal plants, besides many are on the verge of extinction. The synthesis of phytochemicals in plants for medicinal purpose can be improved through various tissue culture techniques and homoeopathic remedies have been found to boost the generation of secondary metabolites. Methodology and Results: A comprehensive review of the scientific literature was carried out by searching through various databases, including Scopus, PubMed, Wiley, Google Scholar. Numerous investigations were conducted to assess the impact of homoeopathic remedies on the growth, phytochemical composition, and essential oil production of various medicinal plants. The most studied homoeopathic preparations were Phosphorus, Sulphur, and Silicea. Homoeopathic preparations were observed to improve photosynthetic rates by distributing greater amounts of photo-assimilated substances in the leaves, ultimately leading to enhanced growth. Homoeopathic medications work on the initial substances like pyruvic acid, ornithine and proline that contribute to the formation of shikimic acid and acetate, while providing the vital energy needed for plants to grow and flourish. These processes generate organic compounds that serve as precursors for various categories of secondary metabolites. Conclusion: Overall, the results suggest that homoeopathic preparations can positively impact the essential oil yield, growth, phytochemical profile of medicinal plants and expanded possibilities for the synthesis of pharmacologically and biologically active compounds. Research into applying homeopathy to grow medicinal plants is highly relevant, as it’s a long-standing practice aiming to optimize production and improve chemical constituent quality.

Keywords: secondary metabolites, homeopathy, medicinal plant, plant tissue culture.
INTRODUCTION

Medicinal plants have remained an integral component of the global healthcare system since the dawn of human civilization. Even though the world’s projected complete flora has not yet been investigated, approximately 10 percent of all plants are thought to have medical properties [1]. The World Health Organisation (WHO) reports that, over 80% of rural people worldwide seek their primary medical care from traditional medicine. Ayurvedic, Homoeopathic, Unani, Naturopathic, Tibetan and even folklore medicines are traditional medical systems that have played a significant role in rural healthcare. The demand for herbal medicine on a global scale is steadily increasing. Usually, plant extracts are utilized for this. Indigenous knowledge of plants and their traditional applications has a major source of knowledge for many commercially used medications [2]. Depletion of medicinal plants is being driven on by increasing population, growing demand for plant products, and illegal trading. Within the global context, the rapid reduction of forest areas is resulting in the extinction of numerous valuable medicinal herbs. Numerous plants containing extremely valuable phytochemicals are challenging to cultivate or may be at risk of extinction due to excessive harvesting. Hence, there is a need to develop techniques that can enhance the production of bioactive compounds and the isolation of phytochemical elements on a large scale through in vitro methods. Improved genetic engineering, particularly transformation technology, along with advancements in tissue culture has created novel possibilities for the mass manufacture of medicines, nutraceuticals, and various compounds beneficial for health and well-being [3]. It has been seen that, manipulating environmental conditions, artificial selection, inducing variant clones and gene transfer technology has the potential to enhance the biosynthetic activity of cultured cells. The utilization of biotechnological tools is pivotal in both the propagation and genetic improvement of medicinal plants. These tools involve methods like in vitro regeneration and genetic modification. Additionally, they can be employed to generate secondary metabolites using plants as bioreactors. The recent advancements in tissue culture technology have surpassed all expectations, enabling substantial growth in research related to the synthesis of plant compounds. It is expected that in the forthcoming years, there will be a significant surge in the commercialization of metabolites and food ingredients derived from plant cell and tissue cultures. The surge in the adoption of plant cell culture systems is a result of deeper understanding of the biochemical and genetic mechanisms governing plant secondary metabolism, as well as the continuous advancements in yield enhancement techniques and the design of large-scale bioreactors [4]. The integration of homeopathy into agricultural practices has instigated a transformative shift from conventional farming methods towards more sustainable and agro-ecological approaches [5]. This transition is accomplished through the utilization of various dynamized homoeopathic preparations while adhering to the prescribed recommendations stipulated in Hahnemann’s Homoeopathic Pharmacopoeia.

MATERIALS AND METHODS

A comprehensive analysis of scientific literature retrieved from databases like Scopus, PubMed, Wiley, and Google Scholar. A total of twelve studies carried out between 2002 and 2021 were selected in the context of this research (Fig. 1). These studies were dedicated to evaluating the effects of homoeopathic remedies on the growth and phytochemical composition of 11 medicinal plant species. In the aggregate, 12 distinct homeopathic formulations at varying dilutions were subjected to analysis.

Secondary metabolites
Plant secondary metabolites are compounds which are produced by plants. They do not serve as primary components in the fundamental life processes of the plants. Nevertheless, they do have a vital function in the plant’s interactions with its surrounding environment [6]. The synthesis of these compounds is profoundly influenced by the physiological and developmental stage of the plant. The production of these compounds is typically less than 1% of the dry weight of the plant [7].

Plant tissue cultures offer an intriguing solution to the challenges of cultivating valuable plants with complex chemical structures. These plants are often hard to grow and at risk of extinction due to overharvesting. Because of their intricate structures and specific chemical requirements, it’s typically too chemicals through traditional chemical synthesis methods. Instead, plant tissue cultures provide a promising alternative for biotechnologically producing important secondary metabolites [8].

**Elicitors**

An "elicitor" is a compound which, when incorporated in small amounts to a living cell system, encourages boosts the production of specific molecules. Elicitors can be categorized based on their "nature" as either abiotic or biotic elicitors or based on their "origin" as either exogenous (derived from pathogens) or endogenous (derived from plants) elicitors [10].

**Figure 1 – Prisma Flow diagram**

**Classification of Secondary metabolites**

Plant secondary metabolites are classified into four groups by the British Nutrition Foundation: Terpenoids: Compounds in this category include cardiac glycosides, sterols, carotenoids, and plant volatiles. Flavonoids, phenolic acids, tannins, coumarins, stilbenes, and lignin are examples of phenolic compounds. Nitrogen-containing substances include cyanogenic glucosides, non-protein amino acids and alkaloids. Compounds containing sulphur include glutathione, glycosylates, phytoalexins, thionins, defensins, and lectins [9].

Biotic elicitors are substances that come from biological sources, including things like plant cell wall polysaccharides (such as...
pectin or cellulose), microorganisms (like chitin or glucans), as well as glycoproteins, G-proteins, or intracellular proteins that interact with receptors and act as biotic elicitors. On the other hand, abiotic elicitors mainly consist of inorganic salts and physical factors that can trigger responses, such as copper and cadmium ions.

Endogenous elicitors, such as galacturonides or hepta-glucosides, are substances that are produced internally within the cell. Exogenous elicitors, on the other hand, are substances like polysaccharides, polyamines, and fatty acids that originate from outside the cell. [11]

**Role of plant tissue culture**

Conventionally, the generation of secondary metabolites required the extensive cultivation of plants on a large scale, leading to a significant demand for land resources. However, this approach had significant limitations. Many economically valuable plants could only thrive in their natural habitats, making them vulnerable to environmental challenges, pathogens, and stress factors. Tissue culture techniques have successfully addressed these problems in the generation of secondary metabolites [12].

**Key methods of plant tissue culture**

Plant *in vitro* technologies, often referred to as tissue culture, involve three fundamental methods. The most frequently employed method entails the cultivation of plant tissue on solidified nutrient media with a gel-like consistency. This approach is primarily employed for crucial processes, with a particular emphasis on regenerating plants from cultured cells. Next in line is the cultivation of plant cells in a liquid suspension, which has demonstrated remarkable effectiveness in regenerating plants through somatic embryogenesis. Another significant technology is meristem culture, developed to address the issue of virus-infected plants. Nowadays, this method is extensively applied to produce crop plants that are free from viruses. Lastly, we have the culture of anthers and microspores, with the aim of producing haploid and homozygous plants, constituting another crucial facet of plant tissue culture (Fig.2) [13].

**Importance of Tissue Culture in producing Pharmaceutical Products of Interest**

The primary benefits of a tissue culture system in comparison to the traditional cultivation of plants are:

1. Valuable compounds can be synthesised under controlled settings, unaffected by the changes in climate or soil conditions.
2. Cells in culture would be free from any microorganisms and pests.
3. Cells from various plant types, whether they are tropical or alpine, can be easily propagated to produce their distinct metabolites.
4. Automated management of cell growth and control of metabolite processes not only reduces labour costs but also significantly enhance productivity [14].
5. Organic substances can be extracted from callos cultures.

Different cell culture techniques are used to produce important bioactive secondary metabolites. Cell suspension cultures, with their fast growth rates, are the preferred choice for large-scale production. Consequently, cell suspensions are used to generate a significant number of cells for qualitative and quantitative growth assessment and producing novel chemicals [15].

**Role of biotechnological approaches for the enhancement of secondary metabolite**

Plant cell culture methods have surfaced as a pragmatic means for both cultivating and delving into the study of plant secondary metabolites. Different *in vitro* techniques, such as elicitation, hairy root culture systems, and suspension culture systems, have been widely used to boost the yield of
secondary metabolites from plants. Suspension culture and elicitation are the main techniques employed to increase secondary metabolite production among these techniques. However, the potential of hairy root cultures and other organ cultures to supply the requirement for secondary metabolites has also been established. Today, advancements in these technologies make it feasible to control and manipulate the pathways responsible for producing plant secondary metabolites with relative ease [16].

**Understanding Metabolic pathways**
Plant tissue culture have a notable impact on uncovering the nutritional needs of plant cells and unlocking their potential to produce secondary metabolites. Initially, researchers focused on the feasibility of generating specific compounds through cell cultures of various plant species. Subsequent endeavours focused on comprehending the factors influencing the synthesis of secondary metabolites, a procedure referred to as optimization. As research progressed, attention shifted towards selecting cell lines that could yield higher quantities of secondary metabolites. Scientists also investigated the enzymes involved in biosynthesis, often utilizing radioactive precursors when deemed necessary to gain insights into these processes.

**Figure 2 - Different ways to obtain a product in vitro.** adapted from [17] Biotechnology: secondary metabolites.

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Role of abiotic elicitors in enhancing secondary metabolites
Abiotic elicitors are substances that originate from non-biological sources and can be classified into physical, chemical, or hormonal factors. Abiotic elicitors, such as salicylic acid, have been shown to enhance the accumulation of specific secondary metabolites, such as salvianolic acid B and caffeic acid, in plant cell cultures. Furthermore, researchers have successfully combined transgenic technology with abiotic elicitors to significantly enhance the generation of secondary metabolites [18].

**Plant growth regulator - Gibberellic acids**
Gibberellic acids, derived from geranylgeranyl diphosphate, are diterpene plant hormones that govern numerous growth and developmental processes. These include seed germination, stem elongation, flowering, fruit maturation, and the regulation of gene expression in the cereal aleurone layer. These gibberellic acids are synthesized by fungus, bacteria, and higher
plants [19]. There are over 100 bioactive gibberellic acids. The primary bioactive gibberellic acids include GA1, GA3, GA4 and GA7.

In the present era, homoeopathic remedies can be applied in tissue culture. These remedies are prepared using a process called potentization, which involves repeated succussions or triturations to activate the therapeutic properties of the drugs. Interestingly, highly diluted homoeopathic medicines have been found to have an effect on plants. Embracing the principle of "Similia Similibus Curentur," homoeopathic medicines have recently gained appeal in the realm of tissue culture.

**Homoeopathy and Secondary Metabolite Synthesis**

Physiological processes (photosynthesis and respiration) involved in the synthesis of the glucose molecule result in the production of organic compounds (sucrose). Carbohydrates, created through photosynthesis, provide the essential carbon skeletons for secondary metabolite formation. Homoeopathic medications work on the initial substances like pyruvic acid, ornithine and proline that contribute to the formation of shikimic acid and acetate, while providing the vital energy needed for plants to grow and flourish. Two significant routes, 1-Deoxy-D-xylulose 5-phosphate (DXP) and mevalonate (MEV), are produced as a result of the precursors. Terpenoids, flavonoids, tannins, alkaloids, phenolic chemicals, these pathways serve as the fundamental basis for a wide array of secondary metabolic categories, including terpenoids, tannins, flavonoids, alkaloids, phenolic compounds, and many others.

**Table 1. An overview of homoeopathic dynamizations improving secondary metabolites.**

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<th>Reference</th>
<th>Summary</th>
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<tr>
<td>Brasileiro B, Leite J, Casali V, Pizziolo V, Coelho O, 2010 [20]</td>
<td>The utilization of various dynamizations of Phosphorus (3cH, 12cH, and 30cH, cH=centesimal Hahnemannian) applied to the soil led to elevated levels of flavonoids in <em>Talinum triangulare</em> (Jacq.) wild plants. These flavonoids are responsible for the antioxidant properties of the plants.</td>
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<tr>
<td>Santos F, Monfort L, Castro D, Pinto J, Leonardi M, Pistelli L, 2011 [21]</td>
<td>During an assessment of the effects of homeopathic medicine Phosphorus at different potencies on the growth and dry biomass of <em>Verbena gratissima</em>, a plant indigenous to Brazil. It was noted that the yields of essential oil production varied between 0.30% and 0.72%. The most noteworthy yield was attained using the 9cH dynamization, exhibiting the highest efficiency in essential oil production at 35.2 mL per unit of dry plant weight. Notably, the 9cH dynamization of Phosphorus led to an increase in the synthesis of compounds such as beta-pineno acetatus, trans-pinocamphone, trans-pinocarvyl and trans-pinocarveol in <em>Verbena gratissima</em> plants.</td>
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<td>Armond, 2005 Cecon and Reis, 2005 [22]</td>
<td>A study was done to assess the growth, essential oil production, and the presence of antimalarial compounds in plant <em>Bidens pilosa</em> L. following the application of 12</td>
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homoeopathic solutions of China. The experimental procedure was conducted with 8 replications and 15 treatments, employing solutions of China in centesimal scale ranging from 2cH to 24cH, along with control groups using 70% ethanol and a mixture of 70% ethanol and distilled water. Notably, the homoeopathic preparations of China in 2cH, 4cH, 6cH, 12cH, 22cH, and 24cH had an impact on the chromatographic constitution of the essential oil extracted from Bidens pilosa L. These preparations resulted in distinctive peaks corresponding to organic acids such as chlorogenic acid, acetylenes, and flavonoids. These compounds are known for their bioactive properties, including antimalarial functions. The potential consequences of applying homoeopathic methods to medicinal plants are socially and economically significant, as they lead to changes in the plant’s phytochemical profiles and an increase in essential oil production.

Castro, 2002 [23]

In a study on Cymbopogon citratus, where homeopathic remedies Sulphur and isotherapeutic 12cH were given, researchers noticed a change in the plant’s phytochemical composition. This shift involved higher levels of neral and geraniol, recognised for their traditional use in relieving spasms and pain.


There was an increase in coumarin levels when the isotherapeutic preparation of Justicia was applied to the leaves and stem of Justicia pectoralis Jacq. This finding provides confirmation that the plant’s vital energy responds to the principle of similitude by channelling its resources towards the production of secondary metabolites with pharmacological activity.

Castro, 2002 [23]

The study examined the impact of homoeopathic solutions on the growth of carrot (Daucus carota) and sugar beet (Beta vulgaris) plants. Phosphorus dynamization, in particular, had a noticeable influence on the growth of carrot and sugar beet plants, with the most significant effects observed near the time of harvest. Lemongrass (Cymbopogon citratus) and chamba (Justicia pectoralis) plants were also part of the study. The homoeopathic treatments involved solutions of humic acid,
isotherapeutic preparations, Sulphur, and a control treatment using water. Diverse factors were evaluated, encompassing the fresh and dry weight of plant material, specific leaf area, water content in ariel parts, and the synthesis of essential oils in the context of lemongrass. The study examined neral, citral, geranial in essential oils and coumarin in methanol extract for these two plants. It found that homoeopathic solutions, especially those with Sulphur, affected the growth, active compound production, leaf structure, and electromagnetic field of lemongrass and chamba plants. Additionally, the effects of these homoeopathic solutions produced discernible peaks, as confirmed in the case of carrot and sugar beet plants.

Duarte, 2003 [24]  
A study was done to assess how Justicia isotherapeutic dynamizations impact the growth and coumarin production in Justicia pectoralis plants. The treatments included dynamizations at various potencies, namely 3cH, 6cH, 12cH, 18cH, 24cH, and 30cH of Justicia isotherapic. These treatments were applied starting from the seedling stage, with the aerial parts of the plants being sprayed at weekly intervals. The quantification of coumarin (a chemical compound) in the samples was performed using high-resolution liquid chromatography. The research found that the fresh matter of leaves and stems, as well as the overall fresh matter and coumarin production, showed variations depending on the specific isotherapeutic dynamization applied. These results confirm the impact of homoeopathic preparations on increasing secondary metabolites.

Carvalho, 2004 [25]  
The use of Natrum muriaticum 2cH in the soil resulted in elevated chlorophyll levels in Tanacetum parthenium leaves, boosting photosynthesis and biomass. This treatment also brought about changes in the photosynthetic rates of Mentha arvensis, which can be attributed to elevated chlorophyll levels. This heightened chlorophyll content enhances light absorption and rubisco activity, consequently facilitating increased carbon absorption. Ultimately, this promotes the production of bioactive molecules with therapeutic significance.
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<th>Author(s)</th>
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<tr>
<td>Bonato C, Proença G, Reis B, 2009 [26]</td>
<td>The effects of homeopathic remedies <em>Sulphur</em> and <em>Arsenicum album</em> on various growth parameters and the essential oil content of <em>Mentha arvensis</em> were investigated. Both drugs were tested in four different dynamizations (6, 12, 24, and 30cH) on the centesimal scale (cH=centesimal Hahnemannian), in addition to a control group treated with water. Among the homeopathic remedies tested, <em>Sulphur</em> demonstrated a more pronounced increase in both fresh and dry biomass compared to <em>Arsenicum album</em>. Both drugs, however, led to an increase in plant height across all dynamizations used. It's worth noting that <em>Sulphur</em> inhibited dry biomass production, except in the case of the 6cH dynamization. Interestingly, <em>Sulphur</em> substantially enhanced the essential oil content in mint plants. <em>Arsenicum album</em> exhibited a similar pattern to <em>Sulphur</em>, with the exception that it increased fresh biomass in the 24 and 30cH dynamizations. These results suggest that both <em>Sulphur</em> and <em>Arsenicum album</em> have the capacity to modify plant metabolism, particularly by promoting secondary metabolism, as evidenced by the increase in essential oil content.</td>
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<td>Verdi, 2016 [27]</td>
<td>A study investigated the impact of homeopathic preparations on the essential oil yield of basil (<em>Ocimum basilicum</em> L.). The treatments included <em>Silicea</em> (30cH, 12cH and 7cH), <em>Equisetum</em> (16cH, 14cH, 12cH) and distilled water as control. Notably, the homeopathic preparation <em>Silicea</em> at 30cH exhibited the most significant increase in essential oil production, with a remarkable 141% enhancement compared to the control group. These results emphasize the potential of homeopathy as a valuable resource in the cultivation of medicinal plants like basil, offering the prospect of significant increase in essential oil production at low expense.</td>
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<td>Ubessi C, Tedesco S, da Silva C, Wagner R, Klein B, Andriolo J, 2021 [28]</td>
<td>A study examined the impact of <em>Phosphorus</em> dynamizations on chamomile essential oil, using hydro distillation and gas chromatography analysis. The results showed increased chamomile oil quantity, yield, and changes in concentrations of key compounds. This research is the first of its kind and implies that homeopathy can be a sustainable method</td>
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Arruda, 2005 [29]

The main objective of this study was to investigate the impact of homoeopathic solutions on the growth, production of bioactive compounds, and leaf structure of *Achillea millefolium* L. The research included various homeopathic remedies in the experiment, such as *Kali carbonicum, Natrum muriaticum, Silicea, Sulphur 3cH*, and a control group treated with water. The study found that the tannin content in the plant was influenced by the homoeopathic solutions. The homoeopathic solutions influenced the chlorophyll content. However, specific details about how each remedy influenced chlorophyll levels were not provided. The flavonoids content in the plant exhibited oscillatory behaviour in response to the homoeopathic solutions. The research concludes that the usage of homoeopathic remedies modified the synthesis of secondary metabolites, notably tannins and flavonoids.

**DISCUSSION**

Plant development, physiological and biochemical responses are influenced by both biotic and abiotic influences. The homoeopathic preparations used in the soil or on the leaves of medicinal plants during cultivation altered the phytochemical spectrum, essential oil production, photosynthesis rates, and the potential for producing active constituents with pharmacological and biological properties [30].

**CONCLUSION**

When homoeopathic preparations were administered during the cultivation of medicinal plants, notable changes were observed in their phytochemical composition, resulting in elevated essential oil yields, accelerated photosynthesis rates and expanded possibilities for the synthesis of pharmacologically and biologically active compounds. These substances have medicinal uses, including antioxidant, anticancer, antispasmodic, and antibacterial activities, among others. Research on homoeopathic application to the *in vitro* cultivation of medicinal plants is very important since it is a long-standing technique that strives to improve both the production and chemical constituent quality. It can boost agro-ecology and strengthen the productivity of medicinal plants. The growing utilization of plant cell culture systems in recent times is likely attributed to an enhanced comprehension of the of secondary metabolite pathways in commercially significant plants. This deeper comprehension of several secondary metabolites in plants has given rise to the field of plant metabolomics, a novel research discipline dedicated to detecting and identifying the biosynthetic routes of these compounds, elucidating their structures, and exploring potential applications. However, our understanding of the biosynthetic processes in plants and cultures is still in the primary stages, necessitating the
development of strategies to expand our insights at the cellular and molecular level.

Acknowledgments

Conflicts of interest
The author(s) declare(s) that there is no conflict of interest.

Statement of financial support
The author(s) declare(s) that this study received no funding.

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[22]. Cecon PR, Reis EL. Essential oil and antimalarial compounds in plants of Bidens pilosa L. treated with the China homeopathy. 2005.


