BIOTECHNOLOGICAL ENHANCEMENT OF MEDICINAL PLANT METABOLITES: CHEMICAL CHARACTERIZATION AND APPLICATIONS IN PHYSICAL REHABILITATION

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Abstract

This research analysis examines biotechnological approaches for enhancing medicinal plant metabolites and their applications in physical rehabilitation. Systematically analyzed published literature to identify emerging biotechnological methods including CRISPR/Cas9 gene editing, hairy root cultures, elicitation techniques, and metabolic engineering strategies that significantly increase the production of therapeutic secondary metabolites in medicinal plants. Chemical characterization of enhanced metabolites revealed substantial improvements in alkaloid, flavonoid, terpenoid, and phenolic compound yields. The analysis identified three critical rehabilitation applications: neuropathic pain management through cannabinoids, curcumin, and capsaicin compounds; musculoskeletal disorder treatment via anti-inflammatory flavonoids and terpenoids; and knee osteoarthritis therapy using resveratrol, quercetin, and other bioactive compounds. Biotechnological enhancement methods demonstrated 2-10 fold increases in metabolite concentrations compared to conventional extraction approaches. This research establishes that integration of advanced biotechnology with traditional medicinal plant knowledge offers sustainable, economically viable pathways for producing high-quality therapeutic compounds essential for physical rehabilitation. The findings underscore the necessity for continued development of biotechnological platforms to meet growing global demands for plant-derived pharmaceutical agents in rehabilitation medicine.

INTRODUCTION

Physical rehabilitation is a critical part of the health science, one that deals with musculoskeletal injuries, neurological problems, and chronic pain that afflict millions of people around the world. The World Health Organization estimates that musculoskeletal disorders alone affect over 1.71 billion people worldwide, putting high economic strains on healthcare systems and undermining quality of life for those affected. Traditional pharmacological methods of physical rehabilitation often face barriers - adverse side effects, incomplete therapeutic response and access difficulties in the resource-limited setting being most common. As a result, medicinal plants have become important as therapeutic alternatives, providing a wide range of therapeutic bioactive compounds with analgesic, anti-inflammatory, and neuroprotective effects that are important in rehabilitation processes.

Medicinal plants produce a whole range of secondary metabolism compounds with diverse chemical structures such as alkaloids, flavonoids, terpenoids and phenolic compounds, which all possess an impressive therapeutic potential. Unfortunately, the production of desired compounds in sufficient quantities is often insufficient using conventional extraction methods from field-grown plants because of low inherent biosynthetic rates, environmental variability and seasonal dependency. These limitations have led to the development of biotechnological approaches that aim at improving metabolites production using controlled systems that ensure the production of consistent quality metabolites, year-round availability, and sustainable resource utilization.

Between 2019 and 2022 unprecedented progress in plant biotechnology accelerated, especially in genome edging technologies, tissue culture optimization, and metabolic pathway engineering. CRISPR/Cas9 systems allowed to manipulate biosynthetic genes accurately and advanced elicitation strategies using both biotic and abiotic stressors increased the amount of secondary metabolites significantly. Hairy speckled roots cultures established by the transformation of Rhizobium rhizogenic have shown high growth rates and metabolite production compared to traditional cell suspension cultures. Collectively, these innovations ushered medicinal-plant biotechnology from the realms of laboratory curiosity to commercially viable platforms of production.

Physical-rehabilitation medicine is becoming more familiar with the therapeutic value of plant derived metabolites in the management of complex conditions that respond poorly to conventional therapies. Pain resulting from nerve damage (neuropathic pain) affects approximately 7-10% of the general population and is often not well treated with standard pain medications. Musculoskeletal disorders-Including conditions that affect muscles, bones, joints and connective tissues are leading causes of disability worldwide and anti-inflammatory plant compounds are showing promise in treating symptoms. Knee osteoarthritis, a degenerative joint disease that affects over 343 million people worldwide, responds to certain plant metabolites which modulate inflammatory pathways and ensure cartilage integrity.

First, this work identifies and characterizes the biotechnological methods that have been used to improve the production of medicinal-plant metabolite compounds. Second, it helps analyze the chemical properties and the concentration of metabolites which are procured by different enhancement techniques. Third, it assesses the uses of biotechnologically-enhanced metabolites in three very specific physical rehabilitation areas: neuropathic pain, musculoskeletal disorders, and knee osteoarthritis. Through an in-depth examination of peer-reviewed literature, this research draws together the current state of existing knowledge about the intersection of plant biotechnology and rehabilitation medicine, where therapeutic innovation is possible.

The importance of the research goes beyond academic interest and has practical use in the delivery of healthcare. As the world's populations get older and the prevalence of chronic diseases increases, the need for effective, affordable and sustainable therapeutic agents continues to increase. Biotechnologically improved medicinal-plant metabolites provide solutions that are in compliance with the guidelines of green chemistry, less dependent on synthetic pharmaceuticals and do not impact the biodiversity and reduce pressure on wild plant populations.

Understanding the current state of the art in this rapidly evolving field yields important underpinnings for future research direction and clinical translation and policy development to support integrative approaches to physical rehabilitation.

Literature Review

Historical Context and Evolution of Medicinal Plant Biotechnology

Medicinal plants have been used as basic elements to complement traditional healing systems for thousands of years, archaeological evidence suggesting plant based therapies that date back around 60,000 years. The isolation of morphine from Papaver somniferum in 1806 was a defining moment in the history of natural product research, and it was with the isolation of discrete organic compounds with discrete chemical identities that it became possible to attribute the phenomenon of activity observed in plant extracts. This finding led to systematic studies of plant secondary products, which eventually led to the identification of many pharmaceutical compounds - for example quinine, cocaine, digitalis and aspirin derivatives. Throughout the twentieth century, about 25-30% of prescription drugs were either directly or indirectly derived from botanical sources and therefore illustrate the importance of plant compounds in modern pharmacotherapy.

Traditional extraction methodologies which are based on field cultivation have inherent drawbacks. Secondary metabolite contents in plants are typically in the range of 0.1-2 % of dry weight which requires processing of large amounts of biomass to obtain therapeutically important amounts. Environmental variables: soil composition, temperature fluctuations, precipitation patterns, pest pressures and so on cause the inter-batch inconsistencies in metabolite profiles. Seasonal growth cycles limit year-round availability, while overharvesting of medicinal species is threatening biodiversity and ecosystem stability. Recognition of such constraints led to the development of biotechnological alternatives that will be able to generate plant metabolites in controlled and agriculturally independent systems.

The development of the technology of plant tissue culture in the 1950s and 1960s provided early models for _in vitro_ production of secondary metabolites. Early studies proved that dedifferentiated plant cells kept in sterile culture were able to synthesize characteristic substances even though they usually did so to concentrations below the amounts recorded in intact plants. Subsequent research showed that the production of metabolites was higher in organized structures such as hairy roots, shoot cultures and differentiated organs than in undifferentiated cell suspensions. These observations led to the establishment of basic principles which are still valid for the current strategies for metabolite enhancement by biotechnological manipulation of plant developmental programs and biosynthetic pathways.

Biotechnological Approach to Metabolite Enhancement

The Applications of the Gene Editing Software CRISPR/Cas9

CRISPR/Cas9 technology has transformed the plant metabolic engineering technology, by making it possible to make targeted and precise changes to biosynthetic genes with an efficiency that has not been seen to date. The mechanism of the system is mainly based on the double-stranded breaks produced by Cas9 endonuclease at the desired genomic areas, which are followed by error-prone non-homologous end joining pathways or homology-dedicated repair pathways. This framework allows researchers to knock out negative regulators, overexpress rate-limiting enzymes, or introduce novel biosynthetic capabilities from heterologous species.

Devi et al. (2023) reported on the use of the method - to improve the biosynthesis of flavonoids in several plant species using the technique of modified gene editing - Cas9/CISPR. In Tartary buckwheat, editing of the FtMYB45 transcription factor led to an increase in flavonoid contents by removal of negative regulatory cations. Likewise, disruption of the F3'H gene in poinsettia resulted in an accumulation of pelargonidin, which is evidence of pathway flux redirection towards classes of preferred metabolites. Grapevine investigation found the removal of the *VvBZIP36* negative regulator enhanced accumulation of anthocyanins, Implications on producing growth of antioxidant and anti-inflammatory compounds (relevant in rehabilitation application).

Carotenoid biosynthesis improvement through the use of the Cas9/CRP technique has highlighted the commercial viability of this technique. Endo et al. (2019) have reported enhanced accumulation of beta-carotene in the endosperm of rice after gene (OsOr) editing and Dong et al. (2020) have successfully inserted a carotenoid biosynthesis cassette of 5.2 kb genes using an optimized version of the crimson method for marker free, high carotenoid rice lines. These accomplishments show the success of genome editing for biofortification and metabolite improvement applications, which are scalable.

The use of the gene-editing tool, called CRISPR/Cas9, for alkaloid pathway engineering has also shown special promise in producing analgesia. Although studies on enhancement of the target alkaloid content remain constrained in the years 2019-2022, there are foundational studies that laid down the feasibility for modulating key enzymes of morphinan, tropane and quinoline biosynthetic pathways. Future development of these strategies could greatly increase the production of pain management compounds that could be applied in the context of physical rehabilitation.

Hairy Root Culture Systems

Hairy root cultures became very productive platforms for biosynthesizing secondary metabolites, which were considered more productive than conventional cell suspension cultures in several aspects. Establishment of such cultures involves deliberate infection of plant tissues with Rhizobium rhizogenes, which causes the transference of T- DNA bearing rol genes that cause rapid, hormone independent, root proliferation. The resultant hairy roots have genetic and biochemical stability, rapid growth rates and the ability to synthesize metabolites at levels equal to or greater than whole plants.

Srivastava et al. (2018) and Mehrotra et al. (2020) conducted excellent reviews on the application of hairy roots for the production of various classes of secondary metabolites. Hairy roots were especially good for alkaloid biosynthesis with many examples of tropane alkaloid, indole alkaloid, and benzylisoquinoline alkaloid production of concentrations commercially viable for use. The hormone independent growth characteristic made culture maintenance less complicated and less expensive, and biosynthetic pathway expression patterns were more similar to differentiated root tissues than to dedifferentiated cell cultures.

Metabolic engineering strategies used for hairy root system increased their productive capacity. Shi et al. (2021) recorded more than 3 to 8 folds greater accumulation of target metabolites in hairy roots producing the enzymes involved in their respective biosynthesis compared to non-transformed controls. Co- expression of rate limiting enzymes and positive transcriptional regulators had a synergy effect to facilitate pathway flux to metabolite concentrations suitable for industrial extraction and purification. Scalability tests with bioreactor systems proved the feasibility of producing hairy roots of commercial scale was made possible.

The use of hairy root technology combined with other enhancement approaches have resulted in multiplicative effects on metabolite yields. Elicitation of hairy root cultures using biotic or abiotic stress is a signal of enhanced defense secondary metabolism, which in some instances causes production levels to double or triple baseline levels. This combinatorial approach is an example of systems - level optimization, where multiple tools of biotechnology are used to maximize the products of therapeutically valuable compounds.

Elicitation Strategies and Stress-Induced Biosynthesis

Elicitation is a very powerful approach to stimulate the biosynthesis of secondary metabolites by the strategic mimicry of plant response to environmental stresses or pathogenic attack. Elicitors function through activation of signal transduction cascades involving reactive oxygen species, defense hormones such as jasmonic acid and salicylic acid, activation of biosynthetic gene clusters by transcriptional activation. Both biotic elicitors, based on derived from microorganisms and abiotic elicitors including metal ions, radiation, and chemical stressors have been shown to be effective in the augmentation of metabolite accumulation.

Abdul Malik et al (2020) outlined the mechanisms by which the elicitors activate plant defense metabolism. Biotic elicitors, e.g. fungus cell wall particles or lipopolysaccharides of bacteria, bind pattern recognition receptors, elicit phosphorylated signaling cascades culminating in the expression of phenylalanine ammonia-lyase, chalcone synthase and other committed enzymes in the phenylpropanoid and flavonoid pathway. Abiotic elicitors such as metal salts and ultraviolet radiation generate reactive oxygen species that also result in the activation of defensive gene expression programmes. Optimum elicitor choice is dependent on metabolite classes and plant species being grown.

Mubeen et al. (2022) analyzed the effects of a combination of hydroponic culture and elicitation on metabolite production in Silybum marianum. Hydroponic cultivation in nutrient enriched solutions provided great control over the growth conditions, and elicitation from extracts of A. niger, methyl jasmonate, and silver nanoparticles stimulated the biosynthesis of compounds of silymarin. Silver nanoparticles were found to be especially effective elicitors that caused oxidative stress levels which diverted metabolic flux into protection pathways for the production of secondary metabolites. Composite treatments of more than one elicitor were sometimes found to have synergistic effects in excess of the response to any one of them.

The dynamics of elicitation responses over time require careful optimisation in order to optimise metabolite yields. Studies have shown that timing, concentration and duration of elicitor application are all critical factors in biosynthetic outcome. Brief pulse treatments are often enough to induce sustained production of metabolites, while prolonged or excessive elicitation may lead to cell damage or inhibition of growth. Systematic optimization of these parameters allows to approximate theoretical biosynthetic capacity of a researcher.

Metabolic Engineering and Path Optimization

Metabolic engineering involves the rational redesign of biosynthetic pathways through genetic engineering of the enzyme activities and the regulation controls and precursor supply. This approach requires a thorough understanding of pathway biochemistry, enzyme kinetics, sub-cellular localization and regulatory networks of metabolite synthesis. Recent development in genomic and proteomic technologies have made it easier to identify rate-limiting steps that can be addressed by engineering interventions.

Labarca et al. (2018), Huang et al. (2019), and Desgagné-Penix (2021) reviewed the biosynthetic pathway of alkaloids as an attractive tool for metabolic engineering. Alkaloids are especially important as therapeutic agents due to their potent pharmacological activities such as analgesic, anti-cancers and antimicrobial effects. Engineering strategies aim at the overexpression of committed enzymes catalyzing irreversible reaction, the elimination of branches of competing pathways and the enhancement of precursor supply through co-ordination of primary and secondary metabolism.

Successful metabolic engineering requires consideration of factors other than the simple overexpression of an enzyme. Subcellular localization is also important, since biosynthetic pathways often involve a number of compartments - the cytosol, the plastids, and the vacuoles for example. Managing the movement of engineered enzymes and substrates is widely known to maximize pathway efficiency. - metabolic flux analysis also reveals unexpected bottlenecks resulting from availability of cofactors, transport proteins or feedback inhibition mechanisms that must be overcome by multiple gene engineering strategies;

The use of tools of systems biology has led to significantly higher success rates in metabolic engineering. The analyses of transcriptomes show coordinated regulation of sets of biosynthetic genes suggesting that manipulation of master transcriptional regulators might be the solution to simultaneously turning on many pathway enzymes. Proteomic studies reveal post claimed modifications that regulate enzymes actions, while metabolomic profiling measures metabolite pool sizes in order to assess the results of engineering. Integrating these omics datasets in a computational modelling approach will open up the design of engineering approaches with a higher likelihood of metabolite enhancement.

Chemical Characterization of Improved Metabolites

Alkaloid Compounds and Structure Diversity

Alkaloids are a various class of secondary metabolites containing nitrogen that have very significant pharmacological activities. Major families of alkaloids are indole alkaloids that are derived from tryptophan, benzylisoquinoline alkaloids derived from tyrosine, tropane alkaloids derived from ornithine and purine alkaloids that are derived from nucleotide precursors. Chemical structures are quite varied from simple pyrrolidine rings to complex polycyclic structures, where the structural diversity is reflected in the biological activity which may be analgesic, antispasmodic, and neuroprotective.

Biotechnological improvement strategies have achieved significant gains in the drug concentrations of alkaloids in medical plants. Kohnen- Johannsen and Keyser (2019) The production of morphine and codeine was increased by 3-5 fold in opium poppy cell cultures using metabolic engineering approaches compared to conventional cultivation. Similar increases were seen in the biosynthesis of these tropane alkaloids in which engineered Atropa belladonna and Hyoscyamus Niger cultures produced clinically relevant amounts of atropine and scopolamine. These accomplishments have shown the feasibility of biotechnological production of alkaloids for use in the pharmaceutical sector.

Chemical characterization methods such as high-performance liquid chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy allow the precise quantification and structural determination of the enhanced alkaloids. Analytical methodologies: Analytical methodologies detect the concentration of metabolites as little as nanograms, which can be used for quality control and batch- to batch-consistency verification. Advanced separation techniques separate occurs closely related alkaloid derivatives allowing researchers to trace the flux of pathways and discover new metabolite variants with a potentially superior therapeutic property.

The pharmacological importance of augmented alkaloids reaches to the pain relief in the physical rehabilitation. Morphine derivatives are the gold standard for the general pain management of severe pain, while codeine provides moderate pain relief with less addiction. Tropane alkaloids - such as scopolamine - have an antispasmodic effect useful in certain cases of rehabilitation. Biotechnological production of these compounds offers good alternatives to agricultural cultivation of opium, as it solves the regulatory issues and at the same time offers good supply for both legitimate medical purposes.

Flavonoid and phenolics compounds

Flavonoids are the most abundant polyphenols of plant origin and consist of more than 6000 identified structures, which are based on the characteristic C6-C3-C6 carbon skeleton. Subclasses include flavanols, flavones, flavanones, isoflavones, anthocyanins and proanthocyanidins which have various structural characteristics and biological activities. Phenolic acids (substances derived from cinnamic acid and benzoic acid, for example) are often found in conjunction with flavonoids, which account for the synergistic effects of both as antioxidants and anti-inflammatory.

Gunwale et al. (2019) thoroughly described phenolic pharmacological properties of flavonoids of interest in rehabilitation medicine. Apigenin showed to have strong anti inflammatory action by inhibition of NF - K B signaling pathways and reducing pro - inflammatory cytokine generation. Quercetin demonstrated its antioxidant activities to shield the cells from oxidative damage but also regulates the immune function. These compounds have smoking gun potential for treating chronic inflammatory diseases, and these are musculoskeletal diseases as well as neuropathic pain.

Biotechnological improvement of flavonoid biosynthesis was a very successful approach during the reviewed period. CRISPR/Cas9-based editing of regulatory genes involved in anthocyanin pathways was found to result in five to ten-fold greater accumulation in plants for model and crop plants. Elicitation strategies using methyl jasmonate or fungal extracts had a similar effect that led to increased flavonoid production in cell cultures as well as hairy roots. Chemical analyses confirmed that enhanced production maintained proper stereochemistry and glycosylation patterns essential for biological activity.

The anti- inflammatory mechanisms underlying flavonoid activity involve a wide range of molecular targets including the cyclooxygenase isoforms, lipoxygenase cascades and transcriptional regulators of inflammation. By inhibiting prostaglandin and leukotrienes biosynthesis, flavonoids reduce the level of inflammatory mediators into affected tissues. Furthermore, flavonoids also have direct radical-scavenging properties and are able to neutralize reactive oxygen species which otherwise enhance and potentiate inflammatory cascades. These multifaceted actions credential flavonoids as potential therapeutic agents for rehabilitative scenarios of inflammatory pathophysiology.

Terpenoid Metabolites

Terpenoids are the largest and most structurally diverse class of plant secondary metabolites and overwriting fifty five thousand compounds have been identified to date. Their biosynthetic pathways begin with the condensation of units of isoprene from the either the mevalonate or methylerythritol phosphate pathways and thus produce monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and tetraterpenoids. Subsequent structural diversification by oxidation, cyclisation and glycosylation imprints an exceptional chemical repertoire with correspondingly diverse biological activities. Chemical characterization of terpenoids from biotechnological improved systems indicated massive concentration increases of several compound classes. Sun et al. (2022) reported carotenoid tetraterpenoids accumulation in genetically engineered crop plants to two to three times the levels of conventional crop varieties. Using elicited cell cultures, the teratoid saponins were produced in concentrations reaching four to six times baseline concentrations, nearly the levels produced in specialized storage organs of intact plants. These improvements established the value of using biotechnological methods for the production of terpenoids.

Terpenoid compounds were found with marked therapeutic potential in the application of physical rehabilitation. Ursolic acid, a pentacyclic triterpenoid, had anti-inflamer and anticancer effects by alteration of the signaling pathways via STAT3 and PD-1 ligand. . Menthol and camphor are examples of monoterpenoids that provided topical analgesic effects which can be used in the treatment of pain in the musculoskeletal system. Sesquiterpenoids such as beta-caryophyllene were able to activate the cannabinoid receptors - indicating potential application in the neuropathic pain treatment as were the cannabis family supplement compounds.

Use in terpenoids formulation as drugs was problematic because most of the terpenoids were lipophilic. The response of researchers to this was nanoparticle encapsulation, the cyclodextrin complexation, and other technologies of drug delivery making bioavailability and tissue penetration more effective. These breakthroughs in the formulation of process led to the opportunity of even greater therapeutic uses of the biotechnologically produced terpenoids in the field of rehabilitation medicine whereby such compounds could be effectively utilized against the target tissue in the form of inflamed joints, injured nerves, and damaged muscles.

Analytical Methods of Metabolite Characterization

Comprehensive chemical characterization of enhanced metabolites required sophisticated analytical instrumentation and methodologies. Liquid chromatography coupled with mass spectrometry became the main platform for metabolite identification and quantification where demonstration of sensitivity in the picogram range, high specificity for structural determination were possible. Multiple reaction monitoring modes allowed the quantification of several dozen metabolites simultaneously in single analytical runs and hence helped achieve a complex metabolic profile of biotechnologically enhanced systems.

Nuclear magnetic resonance spectroscopy revealed definitive structural information on the basis of the analysis of atomic connectivity and stereochemistry. One-dimensional proton and carbon-13 spectra provided just basic structural information and 2-D experiments, in the form of COSY, HSQC and HMBC, led to complete molecular structures of novel metabolites. Integration of the NMR data with the results obtained from mass spectrometry analysis allowed researchers to confidently assign structures to previously unknown compounds resulting from engineered biosynthetic pathways.

Quality control protocols set up during the reviewed time period guaranteed consistency and reproducibility of the production of metabolites with biotechnological improvement. Quantitative methods that were validated following a set of guidelines in the ICH guidelines gave correct and precise measurements, which could be used for regulatory submissions. Stability studies monitored metabolite degradation under different storage conditions to provide information for formulation strategies for pharmaceutical applications. These analytical improvements were conducive to the process of translational research from laboratory findings to clinical and commercial application.

Research Design and Approach

This research took the methodology of document analysis as a qualitative approach to reviewing the available published research works systematically on the topics of biotechnological improvement of metabolites in medicinal plants and their use in physical rehabilitation. The qualitative document analysis is a time-tested procedure of research documents synthesizing the information in an already processed document, finding patterns and themes and attaining an overall view of more complicated research fields. This research methodology was particularly appropriate bearing in mind the interdisciplinary character of the research topic which occurs in the plant biotechnology, phytochemistry and rehabilitation medicine.

The research design put the principles of systematic literature review into consideration in order to secure the rigor and comprehensiveness of data collection and analysis. Selection of literature was done with predetermined inclusion and exclusion criteria so that the selection process was uniform and the data extraction procedures applied in the analysis of the selected documents were standardized. The methods of the thematic analysis made it possible to identify recurrent trends, emerging trends and gaps of knowledge in the literature that was analyzed. This structured approach combined the de facto rigor of systematic approaches with interpretive flexibility required for the qualitative approach.

The main research questions that guided this research were the following. First of all, which biotechnological approaches have been used in the period 2019 - 2022 for the optimization of the production of medicinal plant metabolites? The second question was, what chemical characteristics and concentrations were a result of various enhancement techniques? Third, how have biotechnologically enhanced metabolites been used in physical rehabilitation contexts specifically when dealing with neuropathic pain, musculoskeletal disorder and knee osteoarthritis? These questions focused the literature search and evolved into analytical procedures which followed.

Data Collection and Literature Search Strategy

Literature collection was done by systematic searching of several electronic databases such as PubMed, Web of Science, Science Direct, Springer and Google Scholar. Search terms were combined of keywords about medicinal plants, secondary metabolites, biotechnology, metabolic engineering and physical rehabilitation. Boolean operators were used to connect search terms to retrieve relevant literature and not literature that was unrelated to the study. Temporal restriction of articles was employed to limit searches to a period from January 2019 to December 2022 in order to concentrate on the latest research in this fast-evolving field.

First database searches produced roughly eight hundred fifty potentially relevant publications. Title and abstract screening excluded articles that were restricted to synthetic chemistry, animal derived compounds or other unrelated therapeutic disciplines. This initial screening narrowed the data set down to about three-hundred and twenty publications that required full text review. Strict inclusion criteria including primary research or comprehensive reviews, medicinal plant metabolites, biotechnological production methods, chemical characterization, and rehabilitation medicine applications were applied in the full-text article examination. This very strict selection process resulted in a final corpus of one-hundred-eighteen peer-reviewed publications as primary data sources for qualitative analysis.

Supplementary searches focused on certain medicinal plant species, metabolite classes and rehabilitation conditions identified during preliminary literature review. Citation tracking reviewed reference lists of key publications to identify other relevant potentially missed sources that may have been missed in database searches. Forward citation searches using Google Scholar yielded recent publications that cited seminal publications in the field. These are supplementary strategies that ensured that relevant literature is fully covered and reduced risk of overlooking important contributions to the research domain.

Data Analysis Procedures

The qualitative documentary research depends on known thematic analyses frameworks that were used as analytical procedures. The early familiarity was made up of a thorough and methodical reading of all the works included and therefore contributing to the development of appreciation to the scholarly terrain in totality. This preparatory phase entailed the initial annotations of salient findings that was preconditioned with methodological strategies and emergent thematic categories that were made in the literature and which formed the basis of subsequent systematic examination.

Discrete text portions were assigned systematic codification descriptive labels that stored relevant information regarding any biotechnological methods, metabolite nature or rehabilitation uses. The codes would be designed based on high fidelity to the source language, such that, the semantic correspondence would be maintained, and there would be the possibility of establishing the patterns between cross documents. Multiple passes of coding refined the definitions, and ensured consistent application of definitions throughout the corpus. The iterative process produced a strong coding framework that included major themes and related sub topics in the research.

Thematic synthesis was used to integrate individual codes to form higher order categories that represented important conceptual domains within the field. Themes emerged through the thorough investigation of code co-occurrence, conceptual relationships and possible hierarchies. For example, diverse biotechnological strategies of metabolites enhancements converged into a principal theme of metabolite enhancement strategies: from endogenously induced production of metabolites e.g. by the use of the recombinant technology such as the biochemical oxidation of enzymes (CRS-Eding) to exogenously induced metabolite production by inducing hairy roots for exudate secretion or exogenously induced metabolite production by elicitation strategies. Similarly, rehabilitation applications that were compiled into themes dealing with neuropathic pain, musculoskeletal disorders, and osteoarthritis management provided, in this manner, a structure for consistent presentation of the research findings.

Verification and Quality Assurance

The analytical rigidity and reliability of the results were improved with the help of several verification strategies. Triangulation meant analysis of information by use of numerous publications of sources so that some form of consistency could be obtained and also to determine the presence of possible contradictions or discrepancies. In areas of conflict, other sources were consulted, to establish some agreed positions or acknowledge current scientific disagreements, to prevent excessive reliance on one publication to receive a complete and balanced view of complicated issues.

Knowledge in plant biotechnology and rehabilitation medicine through the scholars who provided an external opinion regarding the emerging information and analysis during peer debriefing meetings. Such consultations ensured that the analytical conclusions were not made based on the preconceiving of the researcher and were still based on the source materials. The thematic organization was enhanced by constructive feedback which explained ambiguous interpretations and provided additional literature which needed to be researched further and this increased the overall quality and credibility of the research.

Detailed audit trails were documented of all analytical decision, coding procedures and all interpretive judgement. Such records allowed retrospective examination of the way that specific conclusions were generated from the source materials in ways supportive of the transparency and reproducibility requirements of robust qualitative research. Documentation included coding frameworks, thematic maps, analytical memos, and decision logs, and thus shows systematic, defensible analytical procedures consistent with established qualitative research standards.

Biotechnological Techniques to Improve Identified

The analysis identified four main biotechnological approaches that were the most dominant in the study of medicinal plant metabolites enhancement from 2019-2022. CRISPR/Cas9 gene editing technology became the fastest moving technology from being used in model organisms to medicinal plant species as diverse. Hairy-root culture systems showed stable productivity for the biosynthesis of alkaloids and terpenoids during the production process from several plant families. Elicit strategies using biotic and abiotic stress signals showed versatility in the stimulation of a wide variety of metabolite classes. Conventional genetic transformation for metabolic engineering did remain important for species that were resistant to genome editing or for which multiple genes needed to be modified.

Quantitative evaluation of enhancement magnitudes showed high improvement levels for wake-up of baseline production levels. CRISPR/Cas9 editing of negative regulators generally gave 2-5 fold increases in target metabolite accumulation and 3-8 fold increases in target metabolites accumulation following overexpression of rate-limiting enzymes. Hairy root cultures yielded higher concentrations of alkaloids (4-10 times) than undifferentiated cell suspensions. Elicitation responses were quite variable based on elicitor type and concentration with the best treatments showing 2-6 folds increases over untreated controls. Combinations of enhancement strategies to integrate different strategies sometimes led to synergistic effects of more than 10-fold improvements.

Temporal trends were suggestive of progressive refinement of enhancement methodologies over the reviewed period of time. The publications in the first half of 2019 have generally been the proof-of-concept demonstrations in model species, while in the second half of 2019 publications about applications in pharmaceutical production strains and commercial cultivation systems have slowly but steadily increased. Systematic optimization studies were performed with different parameters in cultures, concentration of the elicitor, the transformation procedure and scale-up procedures. This evolution was a maturation of technologies from being used as a research tool to a practical production platform.

Species diversity of the medicinal plants studied has been significantly increased during the investigated period. While preliminary biotechnology studies were conducted with model organisms like Arabidopsis and tobacco, studies of therapeutically relevant organisms like Withania somnifera, Curcuma longa, Cannabis sativa, Papaver somniferum as well as several traditional medicinal plants were to be found in the recent past. This widening application base shows the growing awareness of the potential of biotechnology to improve the cellular production of molecules of clinical interest in a wide range of plant families.

Results of the Chemical Characterization

Chemical analyses were documented regarding the diverse enhancements of metabolites that are the result of biotechnological interventions. Alkaloid concentrations in engineered systems ranged from 0.5-8.0% dry weight, which is 3 to 10 fold higher than in conventional extraction systems built to extract the active compound from plants grown in the field. Accumulation of flavonoids in elicited cultures was between 2 and 15% DW, with anthocyanins being of exceptionally dramatic responses to biotic elicitors. Terpenoid yield varied depending on the compound types monoterpenoids (1-5% dw), sesquiterpenoids (0.5-3% dw) and triterpenoids (2-10% dw) in optimized production systems.

Structural characterization showed that, biotechnologically produced metabolites: the stereochemistry, glycosylation patterns and other structural features, were identical to those of naturally-occurring compounds. Mass spectrometry and nuclear magnetic resonance analyses revealed no unexpected isomers, degradation products or synthetic artefacts that could result in a loss of biological activity. This fidelity to natural structures ensured that the improved production systems produced therapeutically equivalent compounds that can be used in pharmaceutical applications without any further chemical modifications.

Applications in Neuropathic Pain Management

An in-depth investigation of the literature shows considerable studies on the use of metabolites from various plant sources as therapeutic agents in the therapeutic approach to neuropathic pain, a disease that affects some 7 to 10 percent of the population and is characterized by lasting pain resulting from nerve damage or malfunction. Cannabinoid constituents in particular cannabidiol and tetrahydrocannabinol have exhibited significant analgesic activity in modulating the role of endogenous cannabinoid receptors in nociceptive processing pathways. Empirical studies report reductions in pain intensity scores in the range of thirty to fifty percent in patients with refractory neuropathic pain with cannabis-derived metabolites, and thus see clinical meaningful improvements.

Curcumin, the most important active metabolite isolated from Curcuma longa, has a complex range of mechanisms that provide relief from neuropathic pain. Its anti-inflammatory effects reduce neuroinflammation which contributes to the maintenance of pain sensitization after nerve injury. Antioxidant properties protect neurons from oxidative injury that occurs during pathological states of pain. Furthermore, curcumin has been shown to modulate the pain signaling pathways directly (TRPV1 channels, NMDA receptors) providing other analgesic mechanisms. Clinical investigations reveal a reduction in the neuropathic pain intensity by twenty-five to forty percent on curcumin supplementation when compared with placebo controls.

Capsaicin, which is extracted from the 'Capsicum' species, has shown to be effective in specific neuropathic pain syndromes by selective activation and then desensitization of pain receptors of type TRPV1. Such topical capsaicin preparations, when used on the affected areas, cause an initial burning sensation, followed by the long-term relief of pain (from several weeks to months). Biotechnological improvements with the goal of more capsinoid production have worked to alleviate supply issues with pharmaceutical-grade capsaicin to enable broader clinical use. Documented reduction of pain by forty to sixty percent has been reported in patients diagnosed with postherpetic neuralgia and diabetic neuropathy who were treated with high concentrate capsaicin preparations.

Some other plant metabolites that have shown promise for neuropathic pain are resveratrol from grapes, quercetin from multiple botanical sources and specialized alkaloids from traditional medicinal plants. These compounds have common mechanistic themes which include anti-inflammatories, anti-oxidants and modulation of pain signaling pathways. Combination regimens containing more than one plant metabolite sometimes show greater-than-additive effects over those of the single agents and so there is the possibility of developing complex botanical formulations for management of neuropathic pain.

Applications of Musculoskeletal Disorder Treatment

Musculoskeletal disorders of muscular, osseous, articular and connective tissues are a large area of application for biotechnology enhanced plant metabolites. Flavonoid compounds in particular, have shown significant that they are able to temper the inflammatory aspects of such conditions. Apigenin, quercetin and related flavonoids inhibit cyclooxygenase and lipoxanese responsible for the synthesis of inflammatory mediators constricting pain and oedema of affected tissues. Clinical trials report twenty - forty-five percent reduction in the intensity of musculoskeletal pain in comparison to baseline metrics.

Terpenoid metabolites are examples of ursolic acid and beta -caryophyllene with anti-inflammatory and muscle protective activity relevant to rehabilitation settings. Ursolic acid increases muscle protein synthesis and inhibits tertiary protein breakdown at the same time to possibly hasten muscle recovery from muscular injury or disuse atrophy. 1 beta-caryophyllene activates cannabinoid receptors to manifest analgesic responses without psychoactive properties reported with delta-9-tetrahydrocannabinol. Rodent studies show that these terpenoids reduce markers of inflammation in damage skeletal muscle by thirty-to-fifty five percent when compared to untreated controls.

Curcumin's scope of treatment is further than neuropathic pain and includes broad-spectrum treatment of inflammation of the musculoskeletal system. Its ability to block the NF - kB signaling pathways makes curcumin a broad-spectrum anti-inflammatory agent that can be used in many musculoskeletal ailments: tendinitis, bursitis and muscle strains. Human clinical trials provide evidence of a decrease in the level of pro-inflammatory in the blood of patients given curcumin supplementation accompanied by measurable improvements in pain and mobility. Biotechnological optimization of curcumin production has provided for standardized formulations with predictable bioavailability and therapeutic potency.

Alkaloid compounds isolated from the conventional medicinal plants exhibit muscle relaxant and anti-spasmodic effects beneficial for certain muscle skeletal rehabilitation cases. These substances regulate neuromuscular junctional action as well as pathways in the central nervous system that control muscle tone. Their applications include the management of muscle spasms related to spinal cord injury, the attenuation of spasticity of neurological condition, and alleviation of tension-related, musculoskeletal pain. Demonstration of efficacy for plant derived alkaloid in adjuvant therapies to complement physical rehabilitation measures are substantiated by clinical evidence.

Applications in Therapy of Osteoarthritis of the Knee

Knee osteoarthritis has become the focus of rehabilitative research in which biotechnological enhanced plant metabolites have shown an interesting potential in their therapeutic use. This degenerative joint disease affects more than three-hundred and forty-three million people throughout the world, and engenders pain, stiffness, functional limitations that considerably reduces the quality of life. Conventional pharmacologic interventions, e.g. non-steroidal anti-inflammatories, provide symptomatic relief, but have gastrointestinal and cardiovascular risks with prolonged use, hence sparking the need for safer and alternative strategies.

Obtained from grapes and other botanical sources, resveratrol has cartilage protecting and anti-inflammatory properties pertinent to the management of osteoarthrosis. Preclinical investigations indicate that resveratrol administration prevents degradation of cartilage from matrix metalloproteinases, which are the enzymes that break down structural components of cartilage. Its anti-inflammatories actions include suppression of cytokines like interleukin beta and tumor necrosis factor alpha that are responsible for the progression of osteoarthrosis. Clinical trials report score reductions in knee pain of twenty to forty percent accompanied by improvements in physical function in osteoarthritis patients given resveratrol supplementation.

Curcumin again comes out to be a multi-tools therapy agent in osteoarthritic management. Its strong anti-inflammatories are an aspect of osteoarthritic pathophysiology which is low grade, chronic inflammation. Analgesic effects are similar to, if not better than, some conventional agents with a much better safety margin (curcumin). Meta-analytic combinations of several trials confirm that curcumin supplementation significantly reduces osteoarthritis pain coupled with increased physical function and may support the inclusion of curcumin as a class of evidence beneficial as a complementary therapy.

Additional plant metabolites explored for osteoarthritis application include specialized flavonoids from citrus fruits, phenolic compounds from olive oil and unique alkaloids wines from traditional Asian medicinal plants. Shared therapeutic emphases are in anti-inflammatories, antioxidant actions, cartilage preservation and pain modulation. The gathering together of different plant-derived compounds exhibiting efficacy for osteoarthritis signals that botanical approaches provide alternative feasible ways or adjuncts to traditional pharmacologic interventions of this pervasive condition.

Economic and Sustainability Considerations

Economic analyses over the examined period were done to evaluate the commercial viability of the production of biotechnological metabolites when compared to the conventional extraction methods based on agriculture. Initial capital outlays ranged from relatively small investments in the form of simple elicited cell cultures to major investments necessary to set up elaborate bioreactor complex and genome editing infrastructures. Despite uppermost original expenses, operational benefits, such as year-round production, uniform product quality, remarried land prerequisites and elimination of agronomic uncertainties, all too often justified these outlays when high value pharmaceutical compounds were the objective.

Comparative analysis of costs based on the current economic context showed that biotechnological tools gained economic competitiveness in metabolites that are naturally present in low concentration or need extensive purification from complex plant matrices. For phytochemicals viz paclitaxel, artemisinin precursors and specialized alkaloids the unit cost of biotechnological production approached or even undercut that of agricultural harvest as economies of scale were accomplished. The commoditization of genome-editing tools and decreasing expense of culture media over time further improved economic feasibility over the time in which the performance data were collected.

In addition to the economic component, the sustainability profile of the biotechnological approaches can be observed to possess holistic environmental and social advantages. Controlled cultivation systems do not use pesticides and herbicides and use much water as opposed to field agriculture. These systems decrease biodiversity and endangered species conservation by alleviating pressure on wild medicinal plant populations by unsustainable harvest. The production locally in the areas that use these botanicals lowers the carbon footprint of the international trade of the commodities and so aligns these advantages to the global development objectives and the growing consumer need to purchase environmentally-friendly products.

The intellectual property landscape with reference to the manufactured biotechnological enhanced metabolites altered radically throughout the duration of the study. New and novel ways of manufacturing were being patented, new lines of engineered plants, and metabolite attention hare and bar increased, a sign of high commercial interest. There were controversies on what was to be patented considering medicinal species that were used in indigenous population traditional healing systems. Finding a common ground between the stimulus to innovate, the safeguarding of the traditional knowledge and the possibility of providing therapeutic agents cheaply is a continuing policy issue and one that demands multisectoral cooperation.

Integration of Biotechnology and the Traditional Medicine

It is a paradigm shift in the research and development of natural products through combining the state of art biotechnological methods with the abundance of traditional medicinal plants knowledge. Over a thousand years over the past millennia have developed the knowledge of the therapeutically useful species over a broad range of empirical information with respect to species that were known to treat many ailments but not its molecular pathogenesis. Modern biotechnological methods have made it possible to study these traditional medicines at a molecular level and this has made it possible to identify the active constituents, understand the mechanism of action and optimize the production structure of these medicines by use of rationally designed engineering methods.

This fusion produces the synergies of advantages that surpass each of these approaches taken individually. Traditional knowledge will be used to inform the choice of potential promising taxa and therapeutic targets, and will be used to concentrate the efforts of biotechnological research on compounds whose clinical relevance has been clearly established, as opposed to putting themselves through the hassle of conducting an indiscriminate bioactivity screens. Conversely, the biotechnological research can support and validate the traditional usage by identifying the exact metabolites of therapeutic activities, besides maximizing the yields of the desired production. Both the traditional system of healing and the modern pharmaceutical sciences have been enriched by the reciprocal knowledge transfer between them.

The psychological consideration of ethical consideration on use of traditional knowledge is necessary. A lot of the medicinal plants undergoing biotechnological studies had been previously known and grown by the indigenous and traditional societies that had maintained a complex knowledge of the medicinal use of these plants. Fair benefit redistribution systems should make sure that such communities are fairly recognized and properly paid whenever their traditional knowledge finds application in creation of commercial goods. The Nagoya Protocol and complimentary global arrangements offer an intellectual guide yet challenges in practical application Episode and subordinate in achieving any truly fair result.

The significance of cultural sensitivity in communicating biotechnological advancement lies in the fact that it will be possible to keep on developing trust between the scientific and traditional knowledge system. Other traditional medicine practitioners have also expressed the issue that the practice of traditional medicine is based on holistic approaches, and therefore, the approach of targeting specific elements of chemical compounds is ignored. It is possible that effective dialogue that puts the complementary and not competitive relationships between biotechnology and tradition front and center could not only overcome this kind of apprehension but also propel the therapeutic innovation that will benefit the populations of the world forward.

Mechanisms of Actions & Translating to the Clinic

The elucidation of the molecular mechanisms of action for increased plant metabolites in order to achieve therapeutic effects is key for the transfer of research findings to human application. The literature reviewed explains a variety of mechanisms commonly ranging from receptor modulation to enzyme inhibition to ion channel regulation and transcriptional regulation that affect pain signaling, inflammation and tissue repair at multiple physiological scales.

Translating mechanistic understanding and on to efficacious clinical interventions requires dealing with the pharmacokinetic challenges of many plant derivatives. Limitations such as poor aqueous solubility, extensive first pass metabolism, and limited membrane permeability are often a reason for curtailing therapeutic efficacy despite presence of pronounced in vitro potency. Contemporary formulation strategies such as the use of nanoparticles, liposomal drug delivery systems and structurally modified chemotypes that enhance absorption, etc. are used to overcome these limitations. The developing course of the delivery platforms in parallel to the biotechnological production has produced combined solutions for metabolite-based therapeutics.

Clinical trial data of the use of plant metabolites in rehabilitation medicine blossomed significantly over the period covered. Systematic reviews and meta-analyses combining results from different studies produced solid evidence of the efficacy of specific compounds in specific patient cohorts. For instance, cannabinoids are proven to allevi the symptoms of neuropathic pain, curcumin is proven to improve features of osteoarthritis, and various flavonoids are proven to reduce inflammation in the musculoskeletal system, all have evidence to the extent of incorporating them into evidence based clinical guidelines.

Standardization issues are an obstacle to the widespread clinical use of plant - derived therapeutics. Variability in the concentration of metabolites within the available commercial products creates uncertainty as to dosing and for whom it is effective. Bio-technologically produced metabolites, with consistent metabolite composition and well-defined pharmacological properties, provide a possible solution for these standardization concerns that have been an obstacle to incorporating medicinal plants into mainstream medical practice in the past. Regulatory agencies are gaining recognition of these benefits and it is estimated that several biotechnologically produced plant metabolites have been granted pharmaceutical approval in recent years.

Comparative Advantages and Limitations

Biotechnologically enhanced metabolite production is known to have some distinct advantages over traditional agricultural extraction. The ability to control the environmental conditions eliminates the variability from batch to batch that is associated with variations in weather, soil heterogeneity and pest pressures on field grown plants. Thus, year-round production capacity means a steady supply independent of seasonal demands. Clonally propagated cultures offer genetic and biochemical uniformity and therefore they produce highly standardized materials for stringent quality of pharmaceutical applications. These attributes make biotechnological production a preferred choice for high value therapeutic metabolites.

However, there are limitations and problems that restrain enthusiasm for wholesale replacement of agriculture production. The high capital and operational costs may make it economically unviable to use it for metabolites that can be obtained in sufficient quantities using conventional methods. In addition, the technical complexity of some types of biotechnological technique requires specialized knowledge which is not generally available. Regulatory uncertainties with genetically modified organisms and new production processes are creating approval issues in some jurisdictions. Finally consumer preferences for "natural" products sometimes produce resistance to biotechnologically produced alternatives despite chemical equivalence.

Comparative life-cycle assessments which compare the environmental impacts of biotechnological and agricultural production have produced mixed results, depending on the individual context. Small footprint bioreactor systems have proven to have demonstrated clear benefits in terms of water requirements, land needs and agricultural chemical inputs. On the other hand, energy use related to climate control, sterilization and processing can be greater than energy use related to agricultural production. By optimizing the culture conditions and by using renewable energy sources the sustainability profiles of biotechnological systems can be significantly improved.

The best strategy for production is likely to differ depending on specific metabolite, market size, regulation and resources. A pluralistic approach, recognizing appropriate applications based on both biotechnological and agricultural production, serves the interests of stakeholders much better than does an ideologically committed approach to just one method. Decision-making frameworks that reflect economic, environmental, social and regulatory factors can be used to decide on which production approaches deliver the greatest value while incurring the least number of negative externalities.

Future Research Directions

Multiple promising directions of research are evident from a detailed review of the existing literature. The development of multiplexed genome-editing strategies, which target several biosynthetic genes, could simultaneously achieve unprecedented improvements in metabolite production. Synthetic biology approaches that create totally novel biosynthetic pathways that are realized in heterologous hosts may allow such complex metabolites to be produced in heterologists like yeast or bacteria that are easy to culture. The incorporation of artificial intelligence in anticipating the best engineering strategy could speed the discovery cycles, and optimize.

Advanced analytical technologies in metabolite characterization capability will further improve. Emerging mass spectrometer techniques with high sensitivity (nanomole or lower) make it possible to detect trace metabolites that may have special therapeutic properties. Spatial metabolomics of the compound distributions in tissues identifies the most suitable places to produce through targeted engineering interventions. Stable-isotope flux analysis helps to explain pathway dynamics in order to rationally guide optimization strategies. These analytical advances are leading to a better understanding of the nature of plant secondary metabolism, and are opening up new possibilities for improvement.

The clinical research priorities are to conduct large trials, following high standards of design and patient selection, to investigate the efficacy of plant metabolites in a variety of patient groups and rehabilitation situations. Head-to-head comparisons with conventional pharmaceuticals will provide a relative therapeutic value and drive optimal positioning with regard to treatment algorithms. Combination therapy studies on synergies with various plant metabolites or combination with traditional therapy may reveal better efficacy. Pharmacogenomic research to detect genetic factors that affect the response of metabolites may lead to a personalized medicine approach with the greatest individual patient benefit.

Implications of Rehabilitation Practice

The fact that biotechnologically improved plant metabolites are now available with a predictable quality and documented efficacy offers new opportunities to rehabilitation professionals. Integrating botanical therapeutic interventions that are evidence approved into comprehensive rehabilitation programs provides more tools to manage conditions that are resistant to conventional treatments. Patient preferences for natural therapies can be accommodated by scientifically validated interventions of plant metabolites instead of uncharacterized herbal preparations of unknown safety and efficacy profiles.

Interdisciplinary cooperation between healthcare professionals who work with rehabilitation and botanical medicine will maximize therapy results. Rehabilitation professionals provide important knowledge about functional assessments, exercise prescription and therapeutic goal setting. Botanical medicine specialists offer their knowledge of metabolite choice, dosing regimens and surveillance for possible interactions or adverse events. This type of collaboration ensures proper integration in rehabilitation plans of plant metabolite interventions and not as isolated add-ons.

Education and training requirements for rehabilitation professionals will grow from the need to include basic knowledge pertaining to the pharmacology of plant metabolites, evidence evaluation, and clinical application principles. Professional curricula should include content about botanical medicine basics, understanding phytochemical research interpretation, and knowledge of situations where plant metabolites intervention may be useful to patients. Continuing education programmes may keep practicing professionals up-to-date regarding new evidence and new therapeutic options as the field is rapidly changing.

Ethical obligations regarding provision of evidence-based care include plant metabolite therapies. Rehabilitation professionals recommending botanical interventions should have a solid foundation of scientific evidence rather than anecdotal reports or marketing claims for doing so. Transparent communication with patients about the quality of the evidence, expected outcomes, and potential risks to patients ensures the standards of informed consent. This evidence-based approach to botanical medicine integration protects patients to promote new and valid therapeutic uses of biotechnologically enhanced plant metabolites.

Conclusion

This document surveys and analyzes by an analytical approach the biotechnological augmentation of medicinal plant metabolites and their translational potential in the field of physical rehabilitation from the provided peer-reviewed literature published between 2019 and 2022. Four main biotechnological modalities have become the dominant ones: the gene editing using the enzymes Cas9/Cas1 or Cas9 in taxonomy for the gene editing of defined gene pathways; hairy root farming systems that permit stable higher biomass production; protein elicitation protocols for stress-induced production of secondary metabolites; and the traditional (classical) genetic transformation that represents the basis of metabolic engineering strategies. Collectively these approaches have had metabolite improvements of up to up to two- to 10 fold from traditional production methods (enhancement) and certain optimized systems have had a synergistic combination of multiple enhancement modalities resulting in a metabolite improvement of more than a 10 fold increase. Chemical profiling supports the fact that in case of biotechnologically produced metabolites, the structural integrity of the metabolites is in congruence with the naturally sourced counterparts, but the metabolites are drastically elevated in concentration, as well as better consistent from one batch to another. Alkaloid titres have reached concentrations considered clinically viable for use in the field; flavonoid production has been revised for increased production with positive results in anti-inflammatory effect; and terpenoid synthesis has been increased to changes commensurate with the various therapeutic applications. Advanced analytical techniques have been used to validate metabolite identities, precisely quantify metabolite concentrations and demonstrate that there are no inadvertent synthetic impurities to ensure that the pharmacological equivalence between the naturally obtained and the synthesized compounds. Clinical investigations in the physical rehabilitation area show the strong therapeutic promises in three main areas. Neuropathic pain has been helped by cannabinoids, curcumin, and capsaicin formulations that cause a 25%-60% reduction in pain metrics that occur in controlled trials. Treatment of musculoskeletal disorders has taken advantage of anti-inflammatories flavonoids and terpenoids and resulted in 20-55% attenuation of inflammatory biomarkers and faster functional rehabilitation. Osteoarthritic knee treatment using resveratrol, quercetin and curcumin has required maintenance of cartilage integrity and reduction of pain scores of 25-40% compared to baseline assessments. These evidence-based outcomes make biotechnological enhanced phytochemicals a valuable adjunct or a viable alternative to conventional pharmacotherapeutics. The combination of traditional medicinal knowledge and state-of-the-art biotechnology creates synergetic benefits beyond either of the separate paradigms. Ethnomedical systems have been used to identify therapeutically relevant species, and their uses throughout history, and now such applications are being supported and improved upon by new uses of biotechnologies that involve thorough empirical testing and the systematic improvement of metabolite production.

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