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Bioactivity-Guided Fractionation Techniques for The Identification and Characterization of Bioactive Compounds from Kashmir Valley Medicinal Herbs

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ABSTRACT

The potential of the medicinal plants in the Kashmir Valley as sources of bioactive chemicals is highlighted by this study, which examines their biodiversity. The research focuses on the systematic identification and characterisation of these chemicals using bioactivity-guided fractionation methods. A variety of biological activities, including antioxidant and antibacterial qualities, are assessed in this study, which starts with plant selection and extraction and continues with fractionation and bioassays. Spectroscopic techniques for structural elucidation verify the chemical identities of bioactive substances, therefore advancing their potential uses in the pharmaceutical and nutraceutical sectors. This study emphasizes the value of sustainable conservation methods and the abundance of natural resources found in the Himalayan area.

Keywords: Bioactivity-Guided, Fractionation Techniques, Bioactive Compounds, Kashmir Valley, Medicinal Herbs, Pharmaceutical, Nutraceutical Industries.

1. INTRODUCTION

The Himalaya has always piqued people's interest and presented intellectual challenges. Among many other benefits, the vegetation offers an endless subject of study. The Himalayan environment's acoustic and aesthetic quality was preserved by the diversity, abundance, and uniqueness of the plant components in their diverse habitats. However, overuse of vegetation, inappropriate land use, natural disasters, and many developmental processes have exacerbated the decline of the Himalaya's biodiversity and healthy ecology in the last several years. Numerous therapeutic plants may be found in the Kashmir Himalayas, one of the most picturesque regions of the Indian Himalayan Region. The temperate, alpine, and sub-alpine regions of the state of Jammu and Kashmir are home to a wide variety of natural plants. In addition to serving as the vital headwaters of the Himalayan rivers and providing habitat for high-altitude fauna, the alpine meadows known locally as "margs" are considered to be nature's home garden, home to an abundance of colorful herbs, medicinal plants, and nutrient-rich grass. Margs are also deeply associated with the livelihoods and religious beliefs of the local people.

One of the Himalaya's most intriguing biomes is the alpine zone. It makes up around 33% of the region's total land area, of which roughly 25.88% is covered in vegetation and the remaining 7.12% is covered with snow all year round. The alpine vegetation consists of snow-swept areas with cushion-shaped plants, herbaceous meadows, bogs, and thickly matted dwarf bushes. India is the sixth most biodiverse country in the world, with a wide range of medicinally significant plant species found there. Approximately 8000 species, or half of all higher blooming plant species in India, are classified as medicinal plants. Given their favorable ecological, phytogeographical, and evolutionary conditions, the Himalayas are acknowledged as one of the world's hotspots for biodiversity. It is a biogeographically distinct area with the greatest level of species endemism in the Asian region. Of the approximately 18,440 plant species found there, 25.3% are indigenous to the area and many of them have therapeutic uses. Both processed finished goods and raw ingredients for medicinal plants are exchanged. As these markets grow and new applications are created, there is a growing demand for a diverse range of species. For the past three years, the majority of Himalayan species of medicinal plants that are marketed on the market have seen an increase in price. Nonetheless, the majority of therapeutic plants utilized by the

industries—roughly 90%—are gathered from the wild. Less than 50 plant species are cultivated commercially, despite the fact that over 800 species are employed in the business.
There are now seventeen Himalayan medicinal plant species recognized in the Indian government's Red Data Book of Indian Plants, and as demand rises, the fear of depletion grows. For populations residing in hilly regions, the gathering and, more lately, the selling of these plants has been a significant source of revenue. The medicinal practices of the Himalayan area are likewise varied. In addition to the Indian Ayurvedic tradition, the region also practices several folk traditions including the Unani (Islamic) and Sowa-rigpa (Tibetan) medical systems. The USA, Japan, and China lead the global market for medicinal plants.

2. LITERATURE REVIEW

Xu, Y., et.al., (2021) The main ingredients of the many oriental formulas used in various traditional medical systems around the world are medicinal plants. Pharmacists are motivated to create novel medications based on the active ingredients or semi-metabolites of medicinal plants that have antituberculosis (TB) capabilities since these plants are a burgeoning supply of medicine. Utilizing both the natural arrangement and the counter TB movement as rules, the counter TB restorative plants remembered for this audit were chosen from the logical writing. The gathered enemy of TB restorative plants were separated into three gatherings: 159 plants that went through top to bottom examination and yielded 335 disconnected compounds; 131 plants whose unrefined concentrates showed hostile to TB action; and 27 plants that were tracked down in distributed works and contained the conventional healers' suggested equation. Our thorough assessment of the restorative plants can help in the advancement of new, more strong enemy of TB drugs.

Naseer, S., et.al., (2017) An endophyte called Cladosporium tenuissimum was disconnected from Pinus wallichiana plants filling in the Lolab Valley of Kashmir, India's Western Himalayas, and its auxiliary metabolite creation was inspected. Three bioactive atoms — scytalone, o-hydroxyphenyl CH3)2CO, and (3S, 5S, 11S)- trihydroxydodecanoic corrosive — were disconnected from the way of life stock utilizing section chromatography. From pandangolide, the stereochemistry of (3S, 5S, and 11S)- trihydroxydodecanoic corrosive was derived. The stereochemistry of pandangolide, which has been secluded from Cladosporium species, is perceived. The cytotoxic and antibacterial capability of the isolated parts was evaluated against types of ATCC 4748, IIIM 25, ATCC 29213, T47D, and MDA-MB-231 cell lines. With an IC50 of 15 µg/ml, (3S, 5S, and 11S)- trihydroxydodecanoic corrosive exhibited remarkable cytotoxic action against the human bosom disease cell line (MCF-7). o-Hydroxyphenyl CH3)2CO shown solid antibacterial movement with IC50 upsides of 23.3µg/ml and 43.7µg/ml against Gram-positive Bacillus cereus IIIM 25 and Gram-negative Escherichia coli ATCC 25922, individually. Interestingly, o-hydroxyphenyl CH3)2CO from a characteristic source is depicted, and C. tenuissimum is the wellspring of (3S, 5S, 11S)-trihydroxydodecanoic corrosive and scytalone interestingly.

Mohammed, S. I., et.al., (2017) Plants and plant-derived metabolites have been used for human health since civilization began. Each of the 400,000–500,000 plant species creates secondary metabolites to survive biotic and abiotic stressors. Alkaloids, steroids, flavonoids, terpenoids, and other plant secondary metabolites have several biological actions. Due to their natural nature, plant-derived metabolites are increasingly sought for medicinal usage worldwide. However, overharvesting medicinal plants for physiologically active secondary metabolites is fast depleting their value. Endophytes, microbial symbionts in plant tissues, mirror the host plant's chemistry. They have garnered global scientific attention due to their variety and capacity to create metabolites comparable to host plants. The recent decade has seen significant study on plant- and endophyte-derived metabolites with antioxidant, antihypercholesterolemic, antidiabetic, and anticancer properties. Recent study on physiologically active metabolites from plant and endophytic fungus is reviewed here. Our lab's endophytic fungal bioprospecting for antihypercholesterolemic compounds is also presented.

Cheema, M. T., (2021) Actinobacteria produce most bioactive substances with broad purposes. In this work, 35 actinobacteria strains were distinguished from Pakistani Himalayan soil tests. Polyphasic scientific classification and natural and synthetic screening found strains with novel metabolites. Antimicrobial viability against Staphylococcus aureus, Micrococcus luteus, Salmonella enterica, Escherichia coli, Mycobacterium aurum, and Bacillus subtilis and anticancer action against PC3 and A549 human disease cell lines were tried Methanolic separates were screened utilizing tender loving care and HPLC-UV/MS. For increase maturation, the actinobacteria strain PU-MM93 was picked for its unmistakable substance profile and cytotoxicity (50-60% development hindrance) against PC3 and A549 cell lines. After increase maturation, sanitization, and underlying explanation, PU-MM93 delivered the cytotoxic anthracycline aranciamycin, aglycone SM-173-B, and neuroprotective carboxamide oxachelin C. Pactamycate, cyclo(L-Supportive of L-Leu), and taurocholic corrosive, the main microbial metabolite announced here, are additionally entrancing. The review exhorted exploring bioactive microorganisms from Pakistan's neglected Himalayas, which can make financially important synthetics.

Jiang, C., et.al., (2021) In order to assess Artemisia absinthium essential oil's (EO) potential utility as a biopesticide for food safety, its chemical profile and phytotoxicity were examined. A. absinthium EO was found to contain 54 different chemicals, the most common of which were eucalyptol (25.59%), linalool (11.99%), and β-myrcene (10.05.%). A combination of three main constituents, EO, and linalool had strong inhibitory action against four recipient species, whereas β-myrcene and eucalyptol had significantly less of an impact. The primary active ingredient responsible for the phytotoxicity of the EO was identified as linalool through the use of bioassay-guided fractionation. The growth of metaxylem and the creation of root hair were dramatically suppressed by linalool, according to a subsequent scanning electron microscope (SEM) investigation. This is the first report on the identification of linalool as the primary active phytotoxic chemical in A. absinthium EO and the clarification of its phytotoxicity mechanism as it relates to alterations in the recipient species' root structures. According to our findings, the EO and its main ingredients may be useful as herbicides that are less harmful to the environment.

3. IDENTIFICATION OF BIOACTIVE COMPOUNDS

In order to identify bioactive chemicals, one must use a methodical and multidisciplinary strategy that incorporates a number of different scientific fields. The following is an in-depth explanation of the procedure:

1. **Selection of Source:** It is possible to get bioactive molecules from a wide variety of natural materials, including plants, marine species, fungus, and microbes that are recognized for their bioactivity.
2. **Extraction:** The compounds are removed from the source material by employing the suitable solvents and extraction procedures (for example, maceration, Soxhlet extraction, and supercritical fluid extraction). The objective of this phase is to separate a wide variety of chemicals from the natural matrix.
3. **Fractionation:** The crude extract is subjected to fractionation in order to isolate and concentrate certain components of interest. Methods include chromatography (such as column chromatography and high-performance liquid chromatography) and a variety of precipitation techniques.
4. **Bioassays:** In order to evaluate the biological activity of isolated fractions or purified substances, bioassays are performed on them. Bioassays are chosen according to the intended activity, which may include any of the following: antioxidant, antibacterial, anticancer, anti-inflammatory, and so on. Quantitative information on the effectiveness and potency of the substances is obtained through these assays.
5. **Structural Elucidation:**Compounds that exhibit considerable biological activity are subjected to structural elucidation, which is the fifth step in the process. In order to ascertain the chemical structure of the bioactive substance and verify its authenticity,

several analytical techniques such as spectroscopy (NMR, MS), X-ray crystallography, and other techniques are utilized.

6. **Characterization:** The physical and chemical characteristics of the bioactive compounds are examined, including their solubility, stability, and toxicity profiles. Characterization is the sixth step in the process. This stage guarantees that a full understanding of the behavior of the compound under a variety of contexts and circumstances is achieved.
7. **The Mechanism of Action:** The importance of having a solid understanding of the biological pathways that the molecule uses to exert its action cannot be overstated. Identifying the molecular targets and pathways that are involved in its biological effects is a necessary step in this process.
8. **Development and Application of Resources:** Following the identification and characterization of a bioactive chemical, subsequent study will concentrate on the possible applications of the compound in many industries, including medicine, agriculture, cosmetics, and others. This may entail the creation of formulations, the testing of efficacy in appropriate models, and ultimately, the marketing of the product.

4. CHARACTERIZATION OF BIOACTIVE COMPOUNDS

In order to identify bioactive compounds and determine their prospective applications, the characterization of these compounds is an essential step. This procedure entails doing a thorough examination of their physical, chemical, and biological features in order to gain an understanding of their behavior and the possible applications they may have. An in-depth look at the procedures involved in character description is as follows:

1. **Characteristics of the Realm:**
   - **Boiling Point and Melting Point:** Figuring out the temperature range at which the compound undergoes a change in state might give valuable information about the molecule's chemical purity and stability.
   - **Solubility:** Determining the appropriate formulations and delivery modalities for the molecule might be aided by determining how well it dissolves in various solvents.
   - **Appearance:** It is possible to obtain preliminary information on the purity and structure of the chemical by observing its appearance, which includes its color, texture, and physical shape (crystalline or amorphous).
2. **Chemical Properties:**
   - **Molecular Weight:** The determination of the molecular weight of the molecule helps provide confirmation of its identification and contributes to the explanation of its structure.
   - **Chemical Structure:** The precise chemical structure may be elucidated via the use of methods such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and X-ray crystallography. This gives significant insights into the content of the substance as well as the arrangement of its atoms.
   - **Functional Groups:** The identification of functional groups from within the chemical, such as hydroxyl and carbonyl, is helpful in predicting the drug's reactivity and the possible biological interactions it may have.
   - **Chemical Stability:** Evaluating the chemical stability of a molecule under different environmental circumstances (such as pH and temperature, for example) allows for the determination of the compound's shelf life as well as its potential for deterioration.
3. **Properties that are biological:**
   - **Bioactivity Assays:** The process of conducting particular bioassays to evaluate biological activities such as antioxidant, antibacterial, anticancer, or anti-inflammatory qualities offers quantifiable data on the effectiveness of the molecule.
   - **Toxicity:** The evaluation of the compound's toxicity through in vitro and in vivo research is helpful in determining its safety profile for prospective therapeutic uses.

4. **Mechanism of Action:**
   - **Target Identification:** Gaining an understanding of the biological targets and pathways via which the chemical exerts its effects is a means of gaining insights into the mechanism of action of the compound.
   - **The Structure-Activity Relationship (SAR):** The process of analyzing how structural alterations impact biological activity is beneficial in terms of optimizing the effectiveness of the substance and minimizing the possibility of adverse effects.
5. **Additional Methods of Product Characterization:**
   - **Spectroscopic Methods:** Methods like as infrared spectroscopy, ultraviolet vi's spectroscopy, and fluorescence spectroscopy are complementary to nuclear magnetic resonance (NMR) and mass spectrometry (MS) in establishing the structure and characteristics of molecules.
   - **Techniques of Microscopy:** Electron microscopy and atomic force microscopy are two examples of techniques that can offer visual information on the morphology of particles and the features of their surfaces.

5. CONCLUSION

The region's tremendous biodiversity and potential for pharmaceuticals are highlighted by the discovery of bioactive chemicals from medicinal plants found in the Kashmir Valley through the use of bioactivity-guided fractionation methods. A wide variety of medicinal plants are found in the Himalayan environment, especially in the Kashmir Himalayas, which are essential to the traditional therapeutic practices and lifestyles of the indigenous people. These priceless resources are threatened by persistent issues including habitat destruction, irresponsible harvesting methods, and rising demand. These plants have tremendous medicinal promise, as evidenced by the methodical discovery and characterisation of their bioactive components, which include anti-inflammatory, antibacterial, and antioxidant properties. Understanding the chemical characteristics and modes of action of these compounds through structural elucidation and characterisation is crucial for their advancement as medicines and nutraceuticals. In order to protect this natural heritage and use its therapeutic advantages for the health and well-being of people everywhere, it will be necessary to go forward with sustainable conservation methods and increased scientific collaboration.

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