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Comparative Analysis of Cytotoxic Effects of Different Solvent Extracts of Lawsonia Inermis and Madhuca Indica, Growing in Sehore, M.P. India

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A B S T R A C T

This study presents a comparative analysis of Mehandi and Mahua bark extracts, examining their cytotoxic effects on HepG2 cells. The research highlights the crucial role of solvent selection and concentration in influencing the observed cytotoxicity. Mehandi bark extracts, obtained through water, ethanol, petroleum ether (PE), and hexane, exhibit concentration-dependent cytotoxicity, with the ethanolic extract consistently outperforming others. The variability in cytotoxic effects is attributed to the differential solubility of bioactive compounds, with ethanol extracting higher concentrations of cytotoxic compounds than water, PE, or hexane. The study suggests the need for further exploration into the specific compounds responsible for Mehandi bark's cytotoxic effects, proposing polyphenols, flavonoids, and tannins as potential candidates. Similarly, the study examines Mahua bark extracts, revealing concentration-dependent cytotoxic effects influenced by solvent choice. The ethanolic extract consistently exhibits the highest cytotoxicity, followed by water, PE, and hexane extracts. The discussion emphasizes the importance of investigating bioactive compounds, suggesting alkaloids, flavonoids, and secondary metabolites as potential candidates. Limitations, including the absence of a control group and statistical analysis, are acknowledged, underscoring the need for careful consideration and validation through in vivo experiments and clinical trials. In conclusion, the comparative analysis of Mehandi and Mahua bark extracts offers valuable insights into their cytotoxic effects, emphasizing the significance of solvent selection and extraction processes. While the results open avenues for potential therapeutic applications, the study's limitations and the complexity of transitioning to clinical settings underscore the importance of cautious interpretation and further validation. This research sets the stage for future investigations into the specific bioactive compounds of both barks, contributing to the understanding of their cytotoxic potential.

Keywords: Sehore, Medicinal plants, Lawsonia inermis, Madhuca indica, Cytotoxic effects, Extracts

1. Introduction

Here Lawsonia inermis, commonly known as henna, is a flowering plant with a rich history of traditional uses and cultural significance. Its leaves have been widely employed for various purposes, including body art, hair dye, and medicinal applications [1,2]. This comprehensive exploration delves into the phytochemistry of Lawsonia inermis and examines its pharmacological effects, shedding light on the diverse bioactive compounds present in the plant and their potential therapeutic applications. The phytochemical composition of Lawsonia inermis is extensive, encompassing a diverse array of compounds with significant biological activities [3]. One of the most well-known constituents of henna leaves is lawsone, a naphthoquinone compound responsible for the plant's characteristic red-orange color. Lawsone possesses antioxidant properties and has been investigated for its potential in various therapeutic applications. Apart from lawsone, henna contains a variety of other secondary metabolites, including tannins, flavonoids, coumarins, and terpenoids [4]. Tannins contribute to the astringent properties of henna and are known for their antioxidant and anti-inflammatory effects. Flavonoids, on the other hand, exhibit antioxidant, anti-inflammatory, and anticancer activities [5]. Coumarins have been associated with antimicrobial and anticoagulant properties, while terpenoids contribute to the plant's fragrance and may possess antimicrobial and anti-inflammatory effects. The presence of polysaccharides, proteins, and amino acids in henna further adds to its phytochemical complexity. These compounds may play a role in various biological activities, including wound healing, anti-inflammatory effects, and immunomodulation.

Lawsonia inermis has demonstrated significant antimicrobial activity against a range of bacteria and fungi. The presence of lawsone, tannins, and terpenoids contributes to its antibacterial and antifungal effects. This antimicrobial activity suggests the potential use of henna in the development of natural antimicrobial agents. The anti-inflammatory properties of Lawsonia inermis have been investigated in various studies. Compounds such as lawsone and flavonoids exhibit anti-inflammatory effects, which may be beneficial in conditions involving inflammation. Additionally, henna has been traditionally used for pain relief, suggesting potential analgesic properties that warrant further exploration. Lawsone, flavonoids, and tannins present in henna contribute to its antioxidant activity. Antioxidants play a crucial role in neutralizing free radicals and oxidative stress, thereby protecting cells from damage. The antioxidant potential of Lawsonia inermis suggests its potential use in preventing or managing conditions associated with oxidative stress. Lawsonia inermis has a history of traditional use in wound healing. The plant's antimicrobial and anti-inflammatory properties, combined with its potential

to promote tissue regeneration, make it a promising candidate for accelerating the wound healing process. This aspect of henna's pharmacological effects may find applications in dermatology and wound care. Studies have explored the immunomodulatory effects of Lawsonia inermis. The plant has shown the ability to modulate immune responses, suggesting potential applications in conditions where immune regulation is crucial. This area of research holds promise for the development of immunomodulatory agents from natural sources. Some investigations have explored the anticancer properties of Lawsonia inermis. Lawsone, flavonoids, and other bioactive compounds present in henna have exhibited cytotoxic effects against certain cancer cell lines. While more research is needed to fully understand its mechanisms and potential applications, these findings highlight the intriguing possibility of henna as a source of anticancer agents. [6-12]

Madhuca indica, commonly known as Mahua, is a versatile plant with a rich history of traditional uses in various cultures. Indigenous to the Indian subcontinent, Mahua has been revered for its economic, cultural, and medicinal significance. This exploration delves into the phytochemistry of Madhuca indica, unraveling the diverse array of bioactive compounds present in the plant, and examines its pharmacological effects, shedding light on its potential therapeutic applications. Madhuca indica is renowned for its intricate phytochemical composition, housing a plethora of bioactive compounds. One of the key constituents is saponins, which contribute to the plant's foaming properties and may have potential therapeutic effects. Another significant class of compounds found in Mahua is flavonoids, known for their antioxidant properties. These compounds play a crucial role in scavenging free radicals and mitigating oxidative stress. [13-16]

Triterpenoids, another group of compounds present in Mahua, have been studied for their potential pharmacological activities, including anti-inflammatory and antimicrobial effects. Additionally, the plant contains fatty acids, glycosides, and various minerals, each contributing to its overall chemical complexity. The combination of these compounds imparts Mahua with diverse biological activities, forming the foundation for its traditional and potential therapeutic uses. Mahua's rich flavonoid content contributes to its antioxidant potential. Antioxidants play a vital role in neutralizing free radicals, protecting cells from oxidative damage. The antioxidant activity of Mahua suggests its potential use in conditions associated with oxidative stress, including certain chronic diseases. Studies have explored the anti-inflammatory effects of Madhuca indica. The presence of triterpenoids, flavonoids, and other bioactive compounds suggests the plant's potential in modulating inflammatory responses. This property makes Mahua a candidate for conditions where inflammation plays a key role. Mahua has demonstrated significant antimicrobial activity against various bacteria and fungi. Saponins and other compounds present in the plant contribute to its antimicrobial effects. This antimicrobial activity aligns with the traditional use of Mahua for treating infections and wounds. Some research has explored the hepatoprotective effects of Madhuca indica. The plant has shown promise in protecting the liver from damage, potentially attributed to its antioxidant and anti-inflammatory properties. This hepatoprotective potential is particularly relevant in the context of liver-related disorders. Mahua has been investigated for its potential antidiabetic effects. Studies suggest that certain compounds in the plant may help regulate blood glucose levels, making it a subject of interest in diabetes research. However, further studies are needed to elucidate the mechanisms involved. Preliminary research indicates potential cardioprotective effects of Mahua. The antioxidant and anti-inflammatory properties of the plant may contribute to cardiovascular health. The exploration of Mahua in the context of heart-related conditions warrants further investigation. Traditional uses of Mahua include pain relief, and recent studies support its potential analgesic and anti-arthritic effects. These properties may be attributed to the anti-inflammatory and antioxidant compounds present in the plant, suggesting its utility in conditions involving pain and joint inflammation. [17-22]

2. Materials and Methods

Collection of the plant material

Collection and Processing of Mehandi and Mahua Bark:

The collection and processing of plant materials, specifically Mehandi and Mahua bark, play a pivotal role in various industries, encompassing cosmetics, pharmaceuticals, and traditional medicine. Situated in Sehore District of Madhya Pradesh, the collection of these barks is deeply rooted in cultural and economic significance. Both Mehandi and Mahua barks are indispensable ingredients in the production of dyes, cosmetics, and traditional medicines.

After collection, the bark is shade-dried to remove excess moisture, preventing mold or fungi growth during storage. The dried bark is then processed into smaller pieces, followed by additional drying in an oven at a low temperature. Grinding the dried bark into a fine powder is the subsequent step, utilizing grinding machines or traditional methods. The resulting powder is stored in dry, airtight containers, away from direct sunlight, ensuring the preservation of potency. These sustainable collection and processing practices are crucial for the long-term viability of Mehandi and Mahua trees and the ecosystems they support, preventing ecological imbalance, habitat destruction, and loss of biodiversity.

Extraction of Mehandi and Mahua Bark

The extraction process of Mehandi and Mahua bark involves a meticulous approach to harness the full spectrum of bioactive compounds present in these plant materials. In the case of Mehandi bark, the extraction process utilizes various solvents, including hexane, ethanol, and petroleum ether (1:3) in a Soxhlet apparatus. This method ensures efficient extraction of compounds with different polarities, including lipophilic components and polar pigments. Hexane targets lipid-soluble compounds, ethanol extracts a wide range of polar compounds, and petroleum ether complements hexane in extracting additional lipophilic components.

For Mahua bark, a similar extraction approach is employed, utilizing hexane, ethanol, and petroleum ether (1:3) with a Soxhlet apparatus. The specific solvents cater to the extraction of lipophilic and polar compounds present in Mahua bark. Notably, water extraction is introduced as an environmentally

friendly alternative, particularly for Mahua, emphasizing the use of a polar solvent. Water extraction facilitates the extraction of water-soluble components, including polyphenols and sugars, providing a holistic approach to harnessing the therapeutic potential of Mahua.

HepG2 Cell Culture and Conditions

HepG2, a vital human hepatocellular carcinoma cell line, serves as a cornerstone in liver-related research, particularly in studies involving drug metabolism and hepatotoxicity. The establishment and maintenance of optimal cell culture conditions are paramount to preserving the viability and functionality of HepG2 cells. Standard culture protocols involve Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and a 1% solution of Penicillin-Streptomycin. Culturing occurs in tissue culture-treated polystyrene dishes or flasks under controlled conditions: 37°C temperature, a 5% CO2 atmosphere, and high humidity. Regular subculture, using trypsin-EDTA for cell detachment, ensures healthy growth. Adherence to detailed record-keeping and protocol adjustments based on experimental needs are imperative for maintaining cell line integrity, ensuring reproducibility, and enhancing the reliability of research outcomes.

MTT Assay for Mehndi and Mahua Extracts on HepG2 Cells

The MTT assay stands as a well-established method for assessing the impact of Mehndi and Mahua extracts on HepG2 cell viability. The assay involves seeding HepG2 cells into a 96-well plate, allowing adherence overnight, followed by exposure to varying concentrations of the extracts. The cells are then incubated for 24 to 72 hours, and MTT solution is added to induce the formation of purple formazan crystals through mitochondrial dehydrogenase activity. After solubilization with dimethyl sulfoxide (DMSO), spectrophotometric measurement at around 570 nm quantifies the formazan crystals' color intensity, reflecting the number of metabolically active, viable cells. This assay provides a quantitative assessment of cell viability and proves valuable in elucidating the potential cytotoxic or protective effects of Mehndi and Mahua extracts at different concentrations, contributing crucial insights to their impact on cellular health in the context of HepG2 cells.

3. Results and Discussion

The comparative analysis of Mehandi and Mahua bark extracts against HepG2 cells provides valuable insights into their cytotoxic effects, emphasizing the importance of solvent selection and concentration. Mehandi bark extracts, obtained using water, ethanol, petroleum ether (PE), and hexane, exhibit concentration-dependent cytotoxicity. The water extract displays moderate cytotoxicity at 40 mg/ml, escalating to the highest observed value of 68 at 640 mg/ml. In contrast, the ethanolic extract consistently outperforms the water extract, starting with a cytotoxicity value of 25 at 40 mg/ml and peaking at 72 at 640 mg/ml (Figure 1). The PE and hexane extracts also demonstrate concentration-dependent cytotoxicity, with the ethanolic extract consistently exhibiting the highest impact. These results underscore the significance of solvent choice, with ethanol extracting potent compounds with pronounced cytotoxic effects.

The observed variability in cytotoxic effects across different solvents may be attributed to the differential solubility of bioactive compounds. Ethanol's ability to dissolve a wide range of molecules might have resulted in the extraction of a higher concentration of cytotoxic compounds compared to water, PE, or hexane. Furthermore, the discussion highlights the need for further investigation into the specific compounds responsible for the observed cytotoxic effects in Mehandi bark. The study suggests polyphenols, flavonoids, and tannins as potential candidates, known for their antioxidant and anticancer properties. Future research should focus on isolating and characterizing these compounds to understand their mechanisms of action and potential synergistic effects.

While the results present promising avenues for exploring Mehandi bark extracts in cancer treatment, the study's limitations are acknowledged. The absence of a control group and statistical analysis makes it challenging to assess baseline cytotoxicity and determine the significance of differences between extracts and concentrations. Additionally, the transition from in vitro studies to clinical applications requires caution, emphasizing the need for further validation through in vivo experiments and clinical trials. The overall findings contribute to understanding the cytotoxic potential of Mehandi bark extracts, paving the way for future research aimed at unlocking their therapeutic applications.

Similarly, the comparative analysis of Mahua bark extracts against HepG2 cells reveals concentration-dependent cytotoxic effects, with the choice of solvent significantly influencing observed cytotoxicity. The ethanolic extract consistently exhibits the highest cytotoxicity, followed by water, PE, and hexane extracts. The water extract displays moderate cytotoxicity at 40 mg/ml, escalating to 73 at 640 mg/ml. In comparison, the ethanolic extract starts with a cytotoxicity value of 30 at 40 mg/ml, reaching the highest observed value of 77 at 640 mg/ml (Figure 2). The PE and hexane extracts also demonstrate concentration-dependent cytotoxicity, with the ethanolic extract consistently exhibiting the highest impact. These findings underscore the importance of solvent selection and highlight the potential therapeutic applications of Mahua bark extracts.

The discussion emphasizes the need for further investigation into the specific bioactive compounds responsible for the observed cytotoxic effects in Mahua bark. Alkaloids, flavonoids, and other secondary metabolites are suggested as potential candidates, necessitating detailed phytochemical analysis and isolation of individual compounds. Understanding the specific mechanisms through which Mahua bark extracts induce cytotoxicity would contribute to harnessing their full therapeutic potential. The limitations of the study, including the absence of a control group and statistical analysis, are acknowledged, emphasizing the need for careful consideration and validation through in vivo experiments and clinical trials before translating these findings into clinical applications.

4. Conclusion

In conclusion, the comparative analysis of Mehandi and Mahua bark extracts provides valuable insights into their cytotoxic effects against HepG2 cells. The concentration-dependent trends and variations observed across different solvents underscore the importance of careful extraction processes and solvent selection. The results open avenues for further research into the specific bioactive compounds responsible for cytotoxic effects in both barks, paving the way for potential therapeutic applications. However, the study's limitations and the complexity of transitioning from in vitro to in vivo settings highlight the need for cautious interpretation and further validation of these promising results.

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