# Quality Audit of Madhuca Longifilia As a Medicinal Herb

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**ABSTRACT**

The present study aimed to conduct a comprehensive quality audit of Madhuca longifolia to authenticate its botanical identity, evaluate its phytochemical composition, and assess its compliance with pharmacopoeial standards for medicinal plant use. The investigation included macroscopic and microscopic characterization of key plant parts such as leaves, bark, root, and ovary, revealing distinct anatomical features necessary for accurate identification. Physicochemical parameters, including moisture content, ash values, and extractive values, were determined and found to be within the acceptable limits set by the Indian Pharmacopoeia, indicating proper handling and quality of the raw material. Preliminary phytochemical screening showed the presence of diverse secondary metabolites such as flavonoids, phenolics, alkaloids, tannins, glycosides, and saponins, particularly in the hydroalcoholic extract. Quantitative analysis confirmed high concentrations of total phenolics (148.6 ± 3.2 mg GAE/g), flavonoids (64.8 ± 2.1 mg QE/g), and tannins (88.4 ± 1.5 mg TAE/g), supporting the plant's pharmacological relevance. TLC profiling using multiple solvent systems enabled the resolution of major compounds, with distinct Rf values and UV absorption maxima confirming the presence of bioactive constituents. Heavy metal testing ensured safety by showing all tested metals below detectable or permissible levels. The findings validate the traditional use of Madhuca longifolia and provide essential parameters for its standardization and quality control, promoting its safe incorporation into modern herbal formulations and pharmaceutical preparations.

**Keywords**

Madhuca longifolia, quality audit, pharmacognostical evaluation, phytochemical analysis, TLC profiling, standardization, herbal medicine, Indian Pharmacopoeia.

## INTRODUCTION

### Botanical Classification and Taxonomy of Madhuca longifolia

Madhuca longifolia, commonly known as Mahua, is a prominent tree species belonging to the Sapotaceae family, which comprises over 1100 species distributed across tropical and subtropical regions worldwide. It is an economically and medicinally significant tree native to the Indian subcontinent, where it has been traditionally used for its diverse therapeutic properties. The species is widely recognized for its pharmacological benefits and extensive applications in Ayurveda, Siddha, and indigenous folk medicine. The correct botanical classification and taxonomy of Madhuca longifolia are crucial for its proper identification, conservation, and sustainable utilization in pharmaceutical and nutraceutical industries.

**Figure 1.1: Madhuca longifolia**

### Taxonomic Diversity and Synonyms

Madhuca longifolia has been subjected to taxonomic revisions over the years due to variations in morphological characteristics, geographical distribution, and genetic differentiation. It is classified into two primary subspecies:

**A. Madhuca longifolia var. longifolia** – Typically found in southern India, this variety exhibits elongated leaves and thrives in well-drained soils.[2]

**B. Madhuca longifolia var. latifolia** – Common in northern and central India, this variety is distinguished by broader leaves and adaptability to diverse climatic conditions.

Additionally, Madhuca longifolia has been historically referred to by various botanical synonyms due to its widespread usage and different regional classifications. Some of the notable synonyms include:

- Bassia longifolia (L.)
- Illipe longifolia (J.F.Gmel.)
- Madhuca indica (J.F.Gmel.)

These taxonomic variations often lead to confusion in scientific literature, herbal medicine formulations, and trade, emphasizing the need for precise botanical authentication to prevent misidentification and adulteration.[3]

**Morphological and Botanical Description**

Madhuca longifolia is a medium to large-sized deciduous or semi-evergreen tree that can grow up to 20 meters in height under favorable conditions. It exhibits a well-developed taproot system, allowing it to withstand dry climatic conditions and making it an essential tree species for afforestation and agroforestry. The morphological features of Madhuca longifolia are as follows:[4]

- **Bark:** The tree possesses a rough, dark brown bark with longitudinal fissures. The inner bark exudes a milky latex, which has been traditionally used for treating skin ailments and inflammatory conditions.
- **Leaves:** The leaves are simple, alternate, and oblong-lanceolate, measuring 10–30 cm in length. They are leathery in texture, with a prominent midrib and secondary veins, arranged in a spiral fashion at the ends of branches. The glossy green surface of the leaves aids in photosynthesis and transpiration regulation.
- **Flowers:** Madhuca longifolia produces creamy-white, fragrant, bell-shaped flowers, which are rich in nectar and attract pollinators such as bees and butterflies. The flowers bloom in dense clusters from February to April, serving as a primary source of sugar-rich nectar used for traditional Mahua liquor preparation.[5]
- **Fruits:** The fruit is a fleshy, ellipsoid berry, turning yellowish-green upon ripening. It encloses one to four seeds, which are rich in fixed oil and bioactive compounds, extensively utilized in the cosmetic and pharmaceutical industries.
- **Seeds:** The seeds contain about 40-50% oil, known as Mahua butter, which has emollient properties and is used in herbal formulations, soap production, and traditional medicine.[6]

**Molecular and Phylogenetic Studies for Authentication**

Advancements in molecular biology and phylogenetics have provided robust tools for authenticating Madhuca longifolia and distinguishing it from morphologically similar species. Techniques such as DNA barcoding, RAPD (Random Amplified Polymorphic DNA), and ITS (Internal Transcribed Spacer) sequencing have been employed to ensure accurate identification. These molecular markers aid in preventing adulteration, which is a common issue in the herbal medicine industry.

**Taxonomical Importance in Herbal Medicine and Pharmacognosy**

Taxonomy plays a vital role in herbal medicine by ensuring the correct identification of medicinal plants, which is essential for maintaining therapeutic efficacy and safety. In the case of Madhuca longifolia, misidentification can lead to the use of substandard or toxic plant materials, reducing its medicinal effectiveness. Regulatory bodies such as WHO (World Health Organization), AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homeopathy), and the Indian Pharmacopoeia Commission (IPC) emphasize the importance of taxonomical classification in herbal standardization.

The correct botanical classification of Madhuca longifolia ensures its sustainable cultivation, conservation, and utilization in pharmaceutical research, traditional medicine, and the nutraceutical industry. With increasing global demand for plant-based medicines, taxonomic authentication is becoming an indispensable tool in maintaining the quality, safety, and efficacy of herbal formulations derived from Madhuca longifolia.

**Traditional and Ethnopharmacological Significance of Madhuca longifolia**

**Historical and Cultural Importance**

Madhuca longifolia has been an essential part of traditional medicine, cultural heritage, and economic practices in South Asia for centuries. Known as Mahua in India, this tree holds immense significance not only in herbal medicine but also in the livelihoods and rituals of many indigenous communities. Historical records indicate that Mahua has been used in Ayurvedic and Siddha medicine for more than a thousand years, primarily for its rejuvenating and healing properties. Ancient texts describe it as a plant of immense medicinal potential, providing therapeutic benefits for a wide range of ailments, including respiratory disorders, digestive issues, skin diseases, and inflammatory conditions.

The cultural significance of Mahua extends beyond its medicinal uses. In many tribal communities, the Mahua tree is considered sacred and is associated with prosperity, fertility, and divine blessings. It is often planted near temples and worshipped as a symbol of abundance. Many rural and indigenous populations depend on Mahua for food, medicine, and economic sustenance. The flowers, which are naturally rich in sugars and nutrients, are used as a primary ingredient in traditional fermented beverages consumed during religious and social ceremonies. This fermented extract is believed to have mild sedative properties and is considered beneficial for relieving stress, improving digestion, and enhancing overall vitality. Additionally, Mahua oil extracted

from its seeds is widely used in traditional lamps and religious rituals, demonstrating its integral role in both medicinal and spiritual practices.

**Medicinal Uses of Different Plant Parts**

The therapeutic properties of Madhuca longifolia have been extensively utilized in traditional medicine, with different parts of the plant serving distinct medicinal functions. The flowers, leaves, bark, seeds, and seed oil are all used to address various health concerns. Each part contains bioactive compounds that contribute to its healing properties, making it one of the most versatile medicinal trees in indigenous healing systems.

The flowers of Mahua are rich in natural sugars, vitamins, and phytochemicals, making them an essential ingredient in traditional herbal remedies. They are commonly used in the treatment of respiratory ailments such as bronchitis, asthma, and persistent coughs, as well as in managing constipation and digestive issues. The high sugar content of Mahua flowers makes them an excellent source of energy, which is why they are often recommended for fatigue and weakness in Ayurvedic medicine. In some regions, Mahua flowers are soaked in water and consumed as a tonic to boost immunity and improve general well-being. Additionally, fermented Mahua flower extract is used as a natural relaxant, helping to relieve stress, anxiety, and sleep disorders.

The leaves of Madhuca longifolia are widely recognized for their anti-inflammatory, antibacterial, and wound-healing properties. Traditional healers commonly prepare leaf poultices to reduce swelling, pain, and infections in cases of injuries, joint inflammation, and skin disorders. Fresh Mahua leaves are crushed and applied topically to treat eczema, boils, and fungal infections. Additionally, leaf decoctions are consumed to regulate blood sugar levels, making them useful in the management of diabetes. The antioxidant and hepatoprotective properties of Mahua leaves have also been explored in scientific studies, further supporting their traditional use in detoxification and liver health.

**Role in Ayurveda and Siddha Medicine**

In Ayurvedic medicine, Madhuca longifolia is classified under the Madhura Rasa (sweet-tasting) category and is considered to have Snigdha (unctuous) and Guru (heavy) properties. Ayurvedic practitioners believe that Mahua helps balance Vata and Pitta doshas, making it particularly beneficial for treating inflammatory conditions, nervous disorders, and metabolic imbalances. Mahua flower formulations are traditionally prescribed to improve liver function, enhance digestion, and support reproductive health. Mahua bark is incorporated into wound-healing formulations, while its seed oil is used to alleviate joint pain and skin ailments.

In Siddha medicine, Mahua is regarded as a powerful rejuvenator that helps strengthen immune function, detoxify the body, and promote overall vitality. Siddha practitioners use Mahua-based formulations to treat respiratory disorders, menstrual irregularities, and neurological conditions. Mahua oil is often prescribed in therapeutic massages for muscle relaxation and pain relief, while Mahua flower extracts are used in nerve tonics to help manage stress and fatigue.

**Ethnopharmacological Studies and Scientific Validation**

The ethnopharmacological importance of Madhuca longifolia has attracted considerable attention from researchers seeking to validate its traditional uses through scientific investigations. Studies have confirmed the presence of flavonoids, saponins, alkaloids, tannins, and triterpenoids in Mahua, which are responsible for its antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and analgesic properties. Research has demonstrated that Mahua flower extracts exhibit significant hepatoprotective effects, supporting its traditional use in liver detoxification and liver disease management. Similarly, Mahua bark extracts have shown strong antibacterial activity, reinforcing their application in infection treatment and wound healing.

Scientific studies on Mahua seed oil have revealed its emollient, anti-aging, and wound-healing benefits, making it a promising ingredient in cosmetic and dermatological formulations. Recent pharmacological research has also focused on Mahua's anti-diabetic, neuroprotective, and anti-arthritic effects, further expanding its therapeutic potential.

**Socioeconomic and Environmental Importance**

Beyond its medicinal applications, Madhuca longifolia plays a crucial role in rural economies, particularly in tribal and forest-based communities. The collection and sale of Mahua flowers, seeds, and oil provide employment opportunities and income generation for many families. In addition, the tree's drought resistance and adaptability make it an excellent candidate for afforestation programs and soil conservation efforts.

However, the sustainability of Mahua is threatened by deforestation, habitat loss, and overharvesting. To preserve this valuable medicinal resource, conservation efforts, cultivation practices, and government policies must be implemented to ensure its long-term availability and integration into modern herbal medicine. The continued research, validation, and standardization of Mahua-based products will help unlock its full potential in pharmaceutical, nutraceutical, and cosmetic industries.

**Geographical Distribution and Habitat of Madhuca longifolia**

**Global and Regional Distribution**

Madhuca longifolia is widely distributed across tropical and subtropical regions, primarily in the Indian subcontinent and Southeast Asia. The tree thrives in a variety of environmental conditions, making it highly adaptable to different ecosystems. It is predominantly found in India, Nepal, Sri Lanka, Bangladesh, Myanmar, Thailand, and Indonesia, where it has been cultivated and

naturally propagated for centuries due to its medicinal, nutritional, and economic importance. Among these regions, India is the largest habitat of Mahua, where it is extensively grown in forested, rural, and agroforestry landscapes.

In India, Madhuca longifolia is commonly found in Madhya Pradesh, Chhattisgarh, Jharkhand, Odisha, Maharashtra, Gujarat, West Bengal, Rajasthan, Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Telangana, and Uttar Pradesh. It is particularly abundant in central and eastern India, where tribal communities have relied on it for generations for food, medicine, and economic sustenance. In northern India, it is found in scattered patches, whereas in southern India, it grows extensively in dry deciduous and tropical moist forests. The tree is also present in the Western Ghats and Eastern Ghats, where it plays a crucial role in supporting biodiversity and soil conservation.

**Habitat and Ecological Adaptability**

Madhuca longifolia is an extremely hardy tree that thrives in diverse environmental conditions, ranging from tropical moist deciduous forests to dry deciduous and semi-arid regions. It is well-adapted to grow in areas with annual rainfall ranging from 500 mm to 1500 mm and can tolerate both drought and seasonal waterlogging. The tree is often found in plains, low hills, riverbanks, and forest margins, indicating its ability to grow in varied topographical conditions.

The ideal habitat for Madhuca longifolia includes open forests, grasslands, mixed woodlands, and degraded lands, where it serves as an important component of the ecosystem. It is highly valued for its soil-binding properties, making it useful in reforestation and erosion control programs. The tree is often planted in community lands, village peripheries, and agroforestry systems, where it provides shade, food, and medicinal resources for both humans and livestock.

**Ecological Role and Biodiversity Conservation**

The Madhuca longifolia tree offers essential ecological functions that support biodiversity maintenance throughout the ecosystem. The nectar-rich tree uses its flowers to draw pollinators including bees and butterflies and birds that pollinate multiple plant species inside its ecosystem. Animals such as bats together with deer and different species of birds depend on the flowers of Madhuca longifolia as their primary dietary source. Many insects along with birds and small mammals use this tree as habitat while its structure provides essential nesting spaces that create benefits for all parts of forest ecosystems.

Packaged leaves and bark of Mahua serve as essential forage items that wild herbivores together with cattle and goats consume in rural areas. The tree serves as a critical factor in preserving soil quality because its presence stops land degradation from occurring. The extended root system helps retain water and recharge groundwater which makes it valuable for regions suffering from desertification and deforestation.

**Major Bioactive Constituents of Madhuca longifolia**

Erichson (1989) illustrates that Madhuca longifolia is a rich source of bioactive phytochemicals that are partially responsible for its tremendous medicinal activity. It is used worldwide in their traditional medicine because it contains a very wide variety of secondary metabolites, including saponins, flavonoids, alkaloids, tannins, terpenoids, steroids, glycosides, and phenolic compounds. The phytochemicals that confer these bioactive compounds are further distributed throughout the plant, including the flowers, leaves, bark, seeds and seed oil, each which contain these phytochemicals in particular doses for different therapeutic benefits. The medicinal potential of Mahua is high because these compounds jointly impart pharmacological effect like anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and analgesic. The bioactive alkaloids present in Mahua are known, however, several of them do occur in alkaloids and most commonly find abode in its bark, seeds and leaves. These alkaloids are responsible for having analgesic, antipyretic, and anti-inflammatory effects and thereby making Mahua very useful to cure diseases related to fever, relief in pain, and inflammation. The alkaloids madhucine and bassic acid from Mahua have shown some promising antimicrobial and neuroprotective properties which further confirms its use in Indian herbal medicine for curing infections and neurological diseases. Additionally, research has also shown that some alkaloids present in Mahua may have anti cancer potential as they seem to prevent tumor cell proliferation and provoke apoptosis in malignant cells.

**Reported Pharmacological Activities of Madhuca longifolia**

Several pharmacological properties of Madhuca longifolia have been widely studied, and the use in Ayurveda, Siddha, and folk medicine is also in agreement with the same. This has various bioactive compounds such as flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and phenolic compounds that contribute to its therapeutic potential. It has been proven by scientific research to be used in effective treatment of inflammatory disorders, microbial infections, metabolic diseases, liver conditions, digestive problems, neurodegenerative disorders, and wound healing. Since the Mahua extracts contain multiple active compounds that modulate oxidative stress, immune responses, enzyme activity and cell signaling patterns, it is a good candidate for herbal drug development. Madhuca longifolia is emerging to be triaged as a natural antiinflammatory, antimicrobial agents, liver protector, pain relief, diabetic management and gastro protector because of its multi target pharmacological effects. The major pharmacological activities of the plant are discussed elaboratively in this and subsequent sections with support of traditional knowledge and scientific validation.

**Anti-Inflammatory Activity**

This has particularly been seen with its anti inflammatory effects which are with arthritis, joint pain, skin inflammation and gastrointestinal disorders and the Madhuca longifolia has shown this positively. The overproduction of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and enzymes (COX-2, iNOS) that causes pain, redness, swelling and tissue damage are associated with inflammatory conditions. Mahua extracts, because of the presence of flavonoids, saponins and triterpenoids contain the

substances which help to modulate these inflammatory pathways by inhibiting the cytokine release, lower the oxidative stress and control immune responses.

Experiments in scientific studies have shown the anti-inflammatory effect of Mahua extracts with the knowledge that they decrease edema and swelling in experimental animal models of inflammation, in which a promise of treating rheumatoid arthritis, osteoarthritis, and inflammatory bowel infection can be also seen. It also becomes further anti-inflammatory due to its inhibition of lipid peroxidation so that chronic inflammation which causes degenerative diseases are not allowed to happen. Given that the majority of NSAIDs (non steroidal anti inflammatory drugs) often results in gastrointestinal side effects, Mahua extracts can be regarded as a natural and safer option to treat chronic inflammatory conditions.

**Antimicrobial and Antifungal Activity**

Madhuca longifolia is a broad spectrum antimicrobial species having activity against both bacterial, fungal and viral infections. It has been reported that extracts of Mahua have special killing rates against Gram positive and Gram negative bacteria like Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae. It is due to the tannins, alkaloids, flavonoids and phenolic acids present that Mahua has

Mahua also has antimicrobial properties though not only antibacterial showing antifungal activity against Candida species, which explains its application in treatment of skin infections, oral thrush and vaginal candidiasis according to its traditional use. Mahua extracts may also have some antiviral potential, though preliminary studies indicate and need further research to determine if it is effective against certain types of viral illnesses. Mahua has antimicrobial effect and therefore can be used as a natural preservative and substitute of the synthetic antibiotics for treating infection.

**Antioxidant Activity**

Madhuca longifolia is a well-known source of strong antioxidants and it is known that oxidative stress is of major importance in chronic diseases, aging and neuro degenerative disorders. Mahua contains the flavonoids, phenolic compounds and terpenoids, which act as powerful free radical scavengers thereby preventing the damage to the reactive oxygen species (ROS). With regards to cardiovascular diseases, diabetes and cancer, antioxidants prevent cellular damage, lipid peroxidation as well as DNA mutations that are underlying causes.

Mahua extracts have been proven in scientific studies to stimulate the action of antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase that are essential anti oxidants which neutralise oxidant stress. Mahua extracts help in improving the cardiovascular health, anti aging and cognitive function leading to its being an acceptable nutraceutical formulation and the ingredient for the anti aging therapies.

**Hepatoprotective Activity**

Traditionally, Madhuca longifolia has been employed as liver tonic and modern research conforms to its hepatoprotective potential. Mahua extract flavonoids and triterpenoid help in the prevention of liver toxicity, aid in the secretion of bile and protects the hepatocytes from hepatic damage caused by alcohol, drugs and environmental toxins. Studies have shown that Mahua extracts lower the elevated liver enzyme levels (ALT, AST, ALP) and enhances the detoxification and lipid metabolism, which will be effective in liver cirrhosis, induced fatty liver disease or H-peptide. Mahua also possesses anti-inflammatory and antioxidant properties that aid the protection of liver, blocking any kind of oxidative stress induced damage, anti-inflammation. Thus, Mahua extracts have been suggested as an important herbal remedy against chronic liver diseases and hepatotoxicity management during liver fibrosis and better liver regeneration.

**Analgesic and Antipyretic Activity**

Other uses of Madhuca longifolia include the treatment of headaches, muscle pain, and fever related diseases due to its natural analgesic and antipyretic (fever reducing) effects. The central and peripheral pain pathways to neurotransmitters playing a role in the mediating of pain (substance P and prostaglandins) are acted upon by alkaloids and flavonoids in Mahua. Mahua extracts have been studied to have the capacity to reduce thermal and mechanical pain sensitivity and can prove to be beneficial in neuropathic pain and chronic pain syndromes. Moreover, Mahua extracts have been observed to decrease the body temperature in fever conditions and can therefore be considered as nontoxic antipyretic agent.

**Anti-Diabetic Activity**

Madhuca longifolia has been reported to possess anti-diabetic properties and thus it may act as a potential natural remedy in the treatment of Type 2 diabetes mellitus. It has been found in research that the Mahua extracts facilitate the decrease in blood glucose levels by increasing the secretion of insulin, improving the intake of glucose, and inhibiting carbohydrate digesting enzymes (α-amylase and α-glucosidase). Better glycemic control, reduced postprandial glucose, better insulin sensitivities are due to these effects. Additionally, Mahua extracts flavonoids and phenolic glycosides protect pancreatic beta cells from oxidative induced damage that is a cardinal feature of the progression of diabetes. Use of Mahua extracts can promote prevention of diabetes complications, ameliorate metabolic function and reduce the risk of insulin resistance.

**Wound-Healing and Skin Regenerative Activity**

Traditional medicine has used Mahua for the treatment of cuts, burns and skin infections. The antimicrobial, anti inflammatory and emollient properties of Mahua extracts helps in the accelerated tissue regeneration, collagen synthesis, and preventing

secondary infection. It is widely used in dermatology and cosmetic formulations for treatment of eczema, psoriasis and dry skin, and therefore, can be referred to as a natural skin healer.

**Recent Advances in Research on Madhuca longifolia**

Madhuca longifolia has gained importance, due to the increasing interest in herbal medicine and medicinal plant with various pharmacological activities. Since the last decade, a number of studies have been carried out relating to its compositional phytochemicals, pharmacological activities and drug formulations. The advancements in biotechnology, pharmacognosy, and the pharmaceutical sciences have provided the researchers with refining of extraction techniques and the isolation of novel bioactive compounds along with improving bioavailability for therapeutic application. The topic covered in this section includes recent trends in research, especially clinical evaluation, biotechnological approach, nanotechnology based drug delivery and global commercialization of Madhuca longifolia.

**Clinical Evaluations and Standardization of Mahua-Based Formulations**

Preclinical studies on the antiinflammatory, hepatoprotective, antimicrobial and anti diabetic properties of Madhuca longifolia have provided strong evidence for its medicinal benefits, but clinical trials and standardization are necessary to integrate it fully into the mainstream healthcare. Studies have recently been made to validate the effectiveness of Mahua based formulations in the treatment of metabolic disorders, inflammatory conditions and liver diseases. In 2022, The clinical study of Mahua flower extract was conducted in India to evaluate the use of Mahua flower extract to treat metabolic syndrome, where it improves lipid profile, blood glucose regulation as well as liver enzyme function and hence, it is a potential natural hepatoprotective and anti-diabetic agent. In another clinical trial, mahua seed oil was proven to have wound healing and skin rejuvenating properties and therefore it was incorporated in dermatological and cosmeceutical formulations.

**Biotechnological Approaches for Enhancing Phytochemical Yield**

Achieving bioactive compound yield increase from Madhuca longifolia through plant biotechnology and genetic engineering has been majorly contributed by. To improve the amount of flavonoids, saponins, and phenolic acids that add to the pharmacological activities of Mahua, tissue culture, metabolic engineering and elicitation methods have been used by researchers. Consequent to this, different tissue culture and micropropagation ways have been developed to grow Madhuca longifolia under controlled situations and in this way guarantee a constant supply of phytochemicals of high quality. Furthermore, elicitor-induced metabolic engineering has already been tried by researchers to enhance medicinal potency of the plant, by using compounds such as jasmonic acid and salicylic acid to promote biosynthesis of secondary metabolites in the plant.

In fact, recent genomic studies have also been able to identify genes that are involved in biosynthesis of therapeutic compounds in Madhuca longifolia. DNA fingerprinting and sequencing are being used to describe the genetic diversity of the plant, so that high yielding plant strains may be selected. However, these advancements will help in sustainable harvesting, conservation strategies, and commercial cultivation so that large scale production of Mahua based phytochemicals can be carried out without harming the natural resources.

**Nanoformulation and Drug Delivery Innovations**

Herbal medicine is still considered as one of the major challenges due to poor solubility and bioavailability of some phytochemicals. Thus, researchers have mobilized nanotechnology based drug delivery systems for enhancing the pharmacokinetics and bioavailability of Mahua derived compounds. Mahua seed oil and flavonoid rich extracts in nanoemulsions and liposomal formulations were tested for topical and systemic applications where absorption is enhanced and the drug is more deeply penetrating in biological tissues. These nanoformulations have been demonstrated as promising for use in the settings of dermatological treatments, anti-inflammatory applications, and pain management therapies.Also, only recent developments have considered the application of polymeric nanoparticles for targeted drug delivery. Researchers have encapsulated bioactive compounds from Mahua extracts within bio degradable nanoparticles to have slow release mechanisms to immobilize anti inflammatory and hepatoprotective agents. These(portions) have major implications for diabetes management, liver disease, and neurodegenerative disease because (these portions) are critical for long term efficacy. Hydrogel based formulations containing Mahua phytochemicals have also been developed for wound healing applications with improved moisture retention and skin hydration along with antimicrobial properties and wound healing, for the treatment of burns, ulcers, chronic wounds etc.

**Future Prospects and Global Market Trends**

Rising demand for natural, plant based medicines and functional foods has led to great interest on Madhuca longifolia as a herbal drug, nutraceutical and cosmeceutical. A large potential exists for incorporation of mahua extracts into herbal capsules, functional beverages and dietary supplements on account of properties of antioxidant, anti inflammatory and metabolic regulation. Mechanical properties of chemical bonds in lignin, cellulose and hemicellulases are investigated by AI computer modelling and computational phytochemistry is conducted to identify Mahua derived compounds with novel therapeutic applications, including anti cancer and neuroprotective treatment.Mahua derived medicines would be the subject of large scale clinical trials to establish the dose optimization, safety profiles, pharmacokinetics in the coming years. Eco friendly extraction techniques are being worked upon by scientists for minimizing environmental impact while at the same time maximizing phytochemical yield. Synergistic formulations, where Mahua phytochemicals are combined with other medicinal plant is another promising area of research. Potential combination therapies against inflammatory disorders, metabolic diseases, and modulation of the immune system were drawn from these formulations.

**Need for Quality Assurance and Standardization**

The necessity to guarantee the safe, effective and standardized herbal products has been increased by the booming demand of herbal medicines and plant therapeutics. Similar to many medicinal plants, Madhuca longifolia shows variability in its phytochemical composition due to environmental, seasonal and geographical factors, and these variables need to be scientifically validated before it can be socially accepted for medicinal use. The term standardization refers to identifying bioactive markers, determining the best extraction method and assure batch to batch consistency meanwhile, term quality assurance is to prevent contamination, adulteration and degradation that might occur during processing and storage. WHO, AYUSH, USP, and ICH have guidelines and analytical techniques that were introduced to ensure the purity, potency and therapeutic reliability of herbal formulations. Therefore, development of measures to strengthen quality control and standardization techniques of Madhuca longifolia for integration into modern medicine and pharmaceutical applications is essential.

**Influence of Environmental Conditions on Phytochemical Variability**

Environmental conditions like changes in the climate, soil properties, altitude and seasonal happenings affect the production of secondary metabolites (flavonoids) in Madhuca longifolia. Synthesis and accumulation of flavonoids, tannins, alkaloids and saponins are directly affected by temperature fluctuations, rainfall patterns, and UV radiation exposure. Plants growing in high altitude areas and increased UV laminations accumulate higher levels of flavonoids and antioxidants due to the fact that they act as a defense mechanism against the environment. Likewise, alkaloids and tannins of Mahua trees increasing in nearly dried up areas are associated in their antimicrobial and astringent properties. Also too, is the availability of nutrients in the soil, especially nitrogen, phosphorus, and potassium, in regulating the biosynthesis of medicinal compounds. Examples include trees of Mahua cultivated in fertile, well drained soils have higher phenolic and saponin content than those grown in nutrient deficients soils whose phytochemical profile is suppressed.

**Impact of Seasonal Changes on Phytochemical Yield**

The major impact on concentration of bio active compounds in Madhuca longifolia is due to the seasonal cycle of plant growth. Peak production of sugars, flavonoids and phenolic acids, and increased levels of tannins and alkaloids occur during the flowering and fruiting stages, whereas no corresponding relationship is found between production of these and tannins and alkaloids during the vegetative growth phase. Flowers grew in early summer have higher concentration of flavonoids than that of flowers collected in monsoon or late autumn, and degraded with moisture, which leaves the bioactive content low for medicinal use. Thus leaves and bark collected during dry seasons have greater tannin and alkaloid retention and hence have better antiand microbial and anti inflammatory effects. By understanding these seasonal variations it is important to time the harvest at the optimal stage for highest therapeutic efficacy in herbal resins.

**Processing Methods and Their Effect on Bioactive Compounds**

It is now well recognized that post harvest processing is fundamental to the chemical integrity and bioavailability of phytochemicals. Specifically, proper drying is very important and the right drying method should be chosen as incorrect drying can cause oxidation, microbial contamination or loss of active compounds. However, sun drying is a traditional process popularly carried out, but its usage with a long exposure to direct sunlight and varying humidity levels leads to significant loss of flavonoids and phenolic acids. Instead, shade drying or controlled low temperature oven drying preserve higher phytochemical levels of heat sensitive phytochemicals. Freeze drying (lyophilization) has been advanced further and these techniques can preserve volatlile compounds and maximize retention of bioactive molecules making them perfect for high value pharmaceutical applications.

**Influence of Extraction Techniques on Phytochemical Potency**

Phytochemical extraction efficiency depends on the method and solvents used to produce yield, purity, and activity stability of those active compounds. Traditional maceration and decoction often have low extraction and lower bioavailability while UAE and SFE are highly efficient and minimal degradation techniques. Polar solvents such as ethanol and methanol are also better at extracting flavonoids, tannins and saponins and non-polar solvents like hexane and chloroform are better at extracting terpenoids and essential oils. Research can optimize selection of solvents used on extraction parameters if possible to achieve maximum recovery of bioactive compounds while preserving pharmacological potency.[54]

**Stability of Phytochemicals During Storage and Shelf Life**

Once extracted, phytochemicals are susceptible to degradation due to environmental exposure such as light, heat, oxygen, and humidity. Essential oils, flavonoids, and phenolic acids are particularly prone to oxidative damage, which can lead to a significant reduction in their antioxidant and therapeutic potential. Research suggests that storing Mahua extracts in airtight, amber-colored glass containers at controlled temperatures helps prolong shelf life and maintain chemical stability. Improper storage conditions, such as exposure to high humidity, can cause the hydrolysis of saponins and tannins, reducing their effectiveness.

**Quality Control Measures for Standardized Herbal Products**

Due to the high variability in phytochemical composition, it is essential to establish stringent quality control measures for ensuring the safety, consistency, and efficacy of Mahua-based medicinal formulations. Standardization involves the identification of chemical markers, optimization of processing techniques, and batch-to-batch consistency testing. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and

nuclear magnetic resonance (NMR) spectroscopy are now widely used to quantify bioactive compounds and detect impurities in herbal extracts. Additionally, regulatory guidelines set by WHO, AYUSH, USP, and ICH emphasize the implementation of Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) to ensure that herbal products meet global safety and efficacy standards.

**Challenges in Achieving Consistency in Phytochemical Content**

One of the primary obstacles in utilizing Madhuca longifolia for large-scale pharmaceutical applications is the inconsistent phytochemical content across different plant batches. Factors such as geographical variation, seasonal changes, and post-harvest handling differences make it difficult to maintain uniformity in bioactive compound concentrations. The lack of standardized cultivation methods further contributes to fluctuations in phytochemical profiles, posing challenges in formulating reproducible and clinically effective herbal medicines. To address this issue, researchers are exploring the potential of plant tissue culture techniques, metabolic engineering, and controlled-environment agriculture (CEA) to produce consistent, high-quality medicinal plant extracts.

**Advancements in Biotechnology for Enhancing Phytochemical Yield**

Recent biotechnological approaches have been implemented to enhance the biosynthesis of key phytochemicals in Madhuca longifolia through tissue culture, genetic modification, and elicitor-induced metabolic engineering. Tissue culture techniques allow for the mass production of medicinal plants under controlled conditions, eliminating variations caused by environmental fluctuations. Additionally, elicitors such as jasmonic acid and salicylic acid have been used to stimulate secondary metabolite production, increasing the yield of flavonoids, alkaloids, and terpenoids. Genomic studies have also identified key biosynthetic pathway genes, paving the way for the development of high-yield plant strains with enhanced medicinal properties. These advancements contribute to sustainable cultivation, conservation, and large-scale commercial production of Mahua-derived phytochemicals.

**Future Directions for Standardization and Global Acceptance**

Madhuca longifolia needs more research to establish it as a widely accepted medicinal plant with validated methodologies of phytochemical standardization and formulation optimization. Optimal dosage and safety profiles, determination of drug interactions, and the integration of this biotherapeutic into contemporary health care systems will be addressing via large scale clinical trials and pharmacokinetic studies. Herbal medicines market is growing quickly at global level owing to demand of plant based therapeutics and functional foods. With the join of scientific research, technological advancement and compliance with the regulation, Madhuca longifolia may be a leading player in evidence based herbal medicine while maintaining its traditional medicinal use.

**Ensuring Consistency in Phytochemical Composition**

Environmental, seasonal and geographical influences inherent to herbal use mean that one of the major challenges is in the inherent variability of phytochemical composition. While the chemical structure of synthetic drugs are known and remains remain consistent from batch to batch, medicinal plants like Madhuca longifolia for instance, release secondary metabolites when conditions change in the field, i.e., soil quality, climate and plant maturities. Medicinal properties can be variable, depending on this natural variability, all of which can affect the efficacy, potency, and safety of herbal formulations. These inconsistencies need to be minimized with the help of the standardization with bioactive marker identification, extraction technique standardization and the consistency of the final herbal product from batch to batch.

**Enhancing Therapeutic Efficacy and Safety**

Herbal medicines work depending on herbals presence of active constituents in optimal concentrations so that formulation will give the expected pharmacological effect. Suboptimal therapeutic effects or unintended toxicity can be generated when phytochemical levels are not standardised, and this is why standardisation is so important for keeping dosages within safe limit. For example, saponins, flavonoid, and alkaloid have been reported as anti inflammatory, hepatoprotective and antioxidant with Madhuca longifolia. The reliability of herbal treatments can be reduced if these compounds have marked variations depending on different batches because they might give varying pharmacological effects.

**Preventing Adulteration and Contamination**

Adulteration, contamination and substitution are one of the biggest issues that the herbal medicine industry faces due to the possibility that the impurities or other molecules may affect the purity and efficacy of the formulation. However, herbal products of commercial availability are unintentionally contaminated with heavy metals, microorganisms, pesticides, and synthetics additive causing a major health risk. Furthermore, when there are scarcity or unaffordability of raw materials, it is normal for the manufacturers to shift from the authentic and economically potent plant to a chemically less active but morphologically most similar plastic plant.

**Regulatory Compliance and Global Market Acceptance**

While herbal medicine is currently enjoying increasing popularity among global healthcare systems, regulatory bodies have thus introduced strict guidelines guaranteeing the quality, safety and efficacy of plant-based therapeutics. However, the World Health Organization (WHO), the United States Pharmacopeia (USP), European Medicines Agency (EMA), and the Indian Ministry of AYUSH have devised rules regarding phytopharmacopoeial standards of medicinal plants i.e. minimum quality requirements of

herbal formulations. The pre-market regulatory guidelines for these herbal medicines include the chemical fingerprinting protocols, toxicity evaluations, pharmacokinetic studies, and batch to batch consistency tests to assure scientific/clinical standards prior to placing these herbal medicines on the world market.

**Advancements in Analytical Techniques for Standardization**

Due to recent advances in the analytical chemistry and biotechnology, new standards for standardization of herbal medicines have emerged. Various modern chromatographic and spectroscopic techniques can be coupled to quantify, identify and quality assess phytochemicals for each herbal medicine batch, and thus ensure that each batch contains consistent phytochemical contents. The phytochemical profile is usually determined by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS); and nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy are used to reveal the molecular structure and active compounds' purity.

**Integration of Standardized Herbal Medicines in Modern Healthcare**

Standardized formulations of herbal medicines are currently being incorporated into evidence based therapeutic approaches in modern healthcare systems as herbal medicines are recognized in modern healthcare systems. Clinical trials are being conducted by many hospitals, pharmaceutical companies, and research institutions to find out the efficacy of herbal extracts in treating chronic diseases, inflammatory disorders and metabolic associated conditions. Integration of standardized herbal formulations into functional foods, dietary supplements, and to alternative medicine are a success and further incorporation into preventive healthcare and wellness management are possible.

**Future Prospects in Herbal Drug Standardization**

The future of the standardized herbal medicines is bright due to continuous advancements in herbal pharmacology and quality assurance. The potential of biotechnologically engineered plant extracts, artificial intelligence based formulation design, and sustainable cultivation practices to improve efficacy, consistency and commercial viability of medicinal plants are being explored by researchers. Such formulations are also being developed for large scale clinical trials, pharmacovigilance programs as well as personalized herbal medicine approaches to ensure that herbal formulations are safe, effective and scientifically validated.

**Regulatory Guidelines for Herbal Drug Quality Control**

As such, the use of herbal medicines in global healthcare industry has grown in volume and has hence prompted the enaction of strict regulatory guidelines that will secure and improve their safety, efficacy, and quality. Unlike the conventional pharmaceuticals, herbal medicines are herbal preparations containing multiple bioactive compounds which tend to make their standardization and quality control a complex issue. Although phytochemical composition is variable, prone to adulteration, and may be contaminated with microbes, and manufacturing is inconsistent, a standard of uniform therapeutic potency and safety remains a challenge. Therefore, these concerns have been addressed by the international regulatory bodies like World Health Organization (WHO), United States Pharmacopeia (USP), European Medicines Agency (EMA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Indian Ministry of AYUSH with the development of complete guidelines relating to the manufacture and quality control of herbal drugs.

**World Health Organization (WHO) Guidelines for Herbal Medicines**

It's through WHO's efforts to set global standards for the quality, safety and efficacy of herbal medicines that electronics retailers can make claims about the quality of their herbs. WHO guidelines provide a guideline for countries to put their national policies and regulatory frameworks for quality control of herbal drugs. Guidelines for the standard practices of cultivation, collection, processing and manufacturing of medicinal plants are established for the purpose of herbal formulation so that they shall exhibit intended pharmacological effects.

The key aspects of WHO's quality control guidelines for herbal medicines include:

- **Good Agricultural and Collection Practices (GACP):** WHO emphasizes the importance of controlled cultivation and harvesting to maintain phytochemical consistency in medicinal plants. This includes proper identification, soil quality management, pest control, drying methods, and storage conditions to prevent deterioration and contamination.

- **Good Manufacturing Practices (GMP):** WHO outlines strict quality control measures in the processing and formulation of herbal drugs, ensuring that batch-to-batch consistency, purity, and potency are maintained. This includes standardized extraction techniques, validated analytical methods, and safety monitoring throughout production.

- **Pharmacovigilance and Adverse Event Monitoring:** WHO recommends a pharmacovigilance system for herbal medicines to monitor adverse reactions, interactions with conventional drugs, and post-market surveillance to ensure consumer safety.

- **Quality Control and Standardization:** WHO specifies the need for quantitative and qualitative testing of active ingredients using techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and thin-layer chromatography (TLC) to verify the identity, purity, and potency of herbal formulations.

- **Microbial and Heavy Metal Testing:** WHO mandates limits for microbial contamination, pesticide residues, and heavy metal content (lead, arsenic, mercury, cadmium) to prevent toxicity and adverse health effects.

**United States Pharmacopeia (USP) and FDA Regulations**

In the USA the regulatory control of herbal products is under the Jurisdiction of Dietary Supplement Health and Education Act (DSHEA) of 1994, which considers herbal preparations as dietary supplements instead of pharmaceutical drugs. The clinical trials undergone by synthetic drugs are not true for herbal medicines, but the manufacturers have to follow FDA guidelines on Good Manufacturing Practices (GMP), ingredient verification and labeling.

- **Authentication of Raw Materials:** Herbal ingredients must be botanically identified to prevent adulteration or substitution with inferior species. DNA barcoding, microscopy, and chromatography are used for species authentication.
- **Quantification of Active Constituents:** Herbal formulations must undergo HPLC, GC-MS, or FTIR analysis to confirm the presence of bioactive compounds in standardized amounts.
- **Microbial and Heavy Metal Limits:** The FDA and USP set permissible limits for microbial contamination, aflatoxins, and heavy metals, ensuring that herbal products are free from harmful pathogens and toxic elements.
- **Labeling and Marketing Claims:** Manufacturers are restricted from making false medical claims about herbal supplements, and labeling must include scientific ingredient names, dosage recommendations, and safety warnings.

While herbal medicines are not classified as prescription drugs in the U.S., compliance with USP monographs, FDA GMP guidelines, and third-party testing enhances consumer trust and market acceptance.

**European Medicines Agency (EMA) and European Pharmacopoeia Standards**

The European Medicines Agency (EMA) has developed stringent regulatory guidelines for herbal medicines, categorizing them into Traditional Herbal Medicinal Products (THMPs) and Well-Established Herbal Medicinal Products (WEHMPs). Unlike dietary supplements in the U.S., herbal medicines in Europe must undergo rigorous scientific evaluation before being marketed.

The European Pharmacopoeia (Ph. Eur.) defines quality control parameters for medicinal plants, ensuring that herbal products meet standardized composition, purity, and stability requirements. Key EMA regulatory guidelines include:

- **Herbal Monographs and Active Ingredient Identification:** The Committee on Herbal Medicinal Products (HMPC) provides herbal monographs specifying approved therapeutic uses, dosage limits, and quality standards for medicinal plants.
- **Clinical and Non-Clinical Testing:** Herbal medicines classified as WEHMPs must undergo clinical trials, toxicological assessments, and pharmacokinetic studies, whereas THMPs can be approved based on traditional use with documented safety records.
- **Heavy Metal, Mycotoxin, and Pesticide Residue Testing:** EMA sets strict maximum allowable limits for toxic heavy metals, fungal contaminants, and pesticide residues, ensuring herbal product safety.
- **Batch-to-Batch Consistency and Stability Studies:** The chemical composition of herbal medicines must be consistent across different batches, requiring rigorous stability testing to determine shelf-life and degradation rates.

**Indian Ministry of AYUSH and Traditional Medicine Regulations**

India is one of the largest producers of Ayurvedic, Siddha, and Unani medicines, and the Ministry of AYUSH has developed Good Manufacturing Practices (GMP) and pharmacopoeial standards to regulate herbal formulations. The Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H) oversees the development of herbal monographs and standardization protocols to ensure the quality of traditional herbal medicines.

AYUSH regulatory requirements include:

- **Authentication and Standardization of Medicinal Plants:** Raw materials must be botanically verified and tested for bioactive compounds.
- **Toxicity and Heavy Metal Limits:** Stringent safety assessments are required for Ayurvedic formulations containing herbal-mineral combinations.
- **Labeling and Compliance with WHO-GMP:** Herbal formulations must be clearly labeled with dosage, ingredients, and safety precautions, ensuring compliance with international guidelines.

The AYUSH ministry continues to collaborate with global organizations to facilitate the acceptance of Ayurvedic and herbal medicines in international markets, bridging the gap between traditional medicine and modern regulatory frameworks.

**Global Harmonization of Herbal Drug Regulations**

As herbal medicine gains worldwide acceptance, regulatory agencies are working toward harmonizing quality control standards to facilitate global trade and clinical research. Efforts by WHO, ICH, and various pharmacopoeial organizations are helping create uniform guidelines for herbal medicine validation, safety monitoring, and therapeutic standardization, ensuring that herbal drugs meet the highest pharmaceutical-grade quality standards worldwide.

**Challenges in the Pharmaceutical Application of Madhuca longifolia**

Despite its extensive pharmacological potential, Madhuca longifolia faces several challenges that limit its widespread adoption in pharmaceutical applications. Issues related to variability in phytochemical composition, authentication difficulties, stability concerns, formulation challenges, regulatory compliance, and commercialization barriers hinder its development as a standardized herbal medicine. Addressing these obstacles is essential for transforming Madhuca longifolia from a traditional medicinal plant into a scientifically validated therapeutic agent.

**Variability in Phytochemical Composition**

The phytochemical content of Madhuca longifolia varies significantly due to environmental and geographical factors, making it difficult to maintain consistency in its medicinal properties. Variability arises from differences in climate, soil quality, altitude, and seasonal changes, which influence the biosynthesis of bioactive compounds such as flavonoids, saponins, tannins, and alkaloids. Harvesting time and post-harvest handling also contribute to fluctuations in phytochemical concentrations, affecting the efficacy of herbal formulations. This inconsistency poses a challenge for standardizing Madhuca longifolia-based pharmaceutical products, as varying phytochemical levels can lead to unpredictable therapeutic outcomes. Implementing controlled cultivation practices and establishing reference standards for active compounds are necessary to minimize this variability and ensure uniform quality in medicinal preparations.

**Issues Related to Authentication and Adulteration**

Authentication of Madhuca longifolia is a significant challenge due to the high likelihood of adulteration and substitution with morphologically similar plant species. Many herbal formulations in the market suffer from incorrect labeling, where inferior or non-medicinal plant species are used as substitutes, leading to reduced therapeutic efficacy. The absence of standardized identification protocols further complicates this issue, increasing the risk of misidentification. Traditional macroscopic and microscopic identification methods are often insufficient to differentiate Madhuca longifolia from other closely related species. Advanced authentication techniques such as DNA barcoding, high-performance liquid chromatography (HPLC), and Fourier-transform infrared (FTIR) spectroscopy are essential for confirming plant authenticity. The development of strict quality control measures and regulatory guidelines for authentication can prevent adulteration and enhance the reliability of Madhuca longifolia-based pharmaceuticals.

**Stability Concerns and Shelf-Life Determination**

The stability of bioactive compounds in Madhuca longifolia is a major concern in pharmaceutical formulations, as many of its phytochemicals are sensitive to environmental conditions such as temperature, light, humidity, and oxygen exposure. Essential oils, flavonoids, and phenolic compounds present in the plant may undergo oxidation or hydrolysis, leading to a decline in therapeutic efficacy over time. Improper storage conditions can also result in microbial contamination, fungal growth, and toxin formation, further reducing the quality and safety of herbal preparations. Establishing optimal storage conditions, protective packaging materials, and stability-enhancing excipients is necessary to maintain the shelf-life of Madhuca longifolia-derived products. Conducting accelerated and real-time stability studies using analytical techniques like HPLC and UV-Vis spectroscopy can help determine the degradation profile of active constituents and develop formulations with improved longevity.

**Formulation Challenges in Developing Standardized Extracts**

Developing pharmaceutical formulations from Madhuca longifolia is challenging due to issues related to solubility, bioavailability, and extract standardization. Many of the plant's bioactive compounds exhibit poor water solubility, limiting their absorption and therapeutic effectiveness in oral dosage forms. Extraction techniques such as solvent extraction, supercritical fluid extraction (SFE), and ultrasound-assisted extraction (UAE) must be optimized to ensure maximum yield and purity of active ingredients. Additionally, the formulation of stable and effective dosage forms such as tablets, capsules, and topical applications requires the selection of appropriate excipients, stabilizers, and bioavailability enhancers. Encapsulation technologies, nanoformulations, and phytosome-based delivery systems have shown promise in improving the solubility and pharmacokinetics of herbal compounds, making them more suitable for pharmaceutical applications. Overcoming formulation-related challenges will allow for the production of more effective and standardized Madhuca longifolia-based medicinal products.

**Regulatory and Compliance Barriers**

The integration of Madhuca longifolia into mainstream pharmaceuticals is hindered by strict regulatory requirements that govern the safety, efficacy, and standardization of herbal medicines. Unlike synthetic drugs, herbal formulations face significant scrutiny due to concerns over inconsistent phytochemical composition, potential toxicity, and lack of clinical validation. Different

countries have varying regulatory guidelines, making it difficult to achieve global acceptance for Madhuca longifolia-based formulations. The absence of well-established clinical trials further limits its therapeutic validation, delaying regulatory approvals. Compliance with Good Manufacturing Practices (GMP), Good Agricultural Practices (GAP), and pharmacopoeial standards is essential to ensure quality assurance and safety. Conducting rigorous toxicological studies, pharmacokinetic evaluations, and randomized clinical trials will help establish Madhuca longifolia as a scientifically validated herbal medicine.

**Limited Clinical Studies and Toxicological Data**

Originally reported in the traditional uses in the Punjab and northern Pakistan, Madhuca longifolia has limited clinical studies and toxicological evaluation and therefore is not well documented about precisely how therapeutic it can be and how safe it is. Its acceptance in evidence based medicine is hampered by the limitation of the scientific research on its pharmacokinetics, pharmacodynamics and long term effect. There is no standardized dosing guidelines, which entails the risk of toxicity, especially with long term use. There are not many review on the herb drug interactions, one of which can pose problems on its compatibility to conventional medications. Critical data of the efficacy, dosage optimization and possible side effects can be obtained through well designed clinical trials and preclinical toxicity studies. The credibility and introduction in pharmaceutical formulations of its therapeutic benefits will be enhanced by establishing standardized protocols for evaluating the same.

**Sustainability and Ethical Harvesting Issues**

Madhuca longifolia is known to be harvested for its use in herbal medicine and this has raised concerns of overharvesting and loss of sustainability. The indiscriminate collection of wild plant populations threatens biodiversity by degrading habitat and $depletion of resources. To have a reliable supply chain without damage to the ecological system, ethical considerations must be made in terms of the buying of sustainable harvested product versus cultivated product. Such practices as encouraging controlled cultivation, agroforestry and organic farming can maintain the quality that is consistent while preserving natural population. Regulating harvesting practices with government policies and conservation efforts of Madhuca longifolia for pharmaceutical applications requires to be done.

**Challenges in Commercialization and Market Penetration**

Madhuca longifolia has many challenges to it being commercialized in the form of pharmaceutical products; it faces competition from well established herbal products, healthcare professional and consumer awareness, and adoption. Madhuca longifolia is deficient in meeting the potential users know of medicinal benefits of the plant and is not demanded in the worldwide market. Furthermore, patent protection on traditional knowledge of herbal unused is, in the absence of this, a barrier to investment in product development and innovation. Slower commercial expansion is also provided by marketing and distribution challenges of branding, regulatory approvals and gaining the trust of the consumers. Promotional strategies should be implemented, public awareness campaigns undertaken and scientific collaborations entered in order to boost its market penetration and awareness among the pharmaceutical industry.

**Extraction Cost and Processing Efficiency**

The major impediment for pharmaceutical use of Madhuca longifolia is the cost of extraction and processing. Usually, the traditional extraction methods have low yield, solvent contamination leading to loss of bioactive compounds. Implementation of new green extraction technology, like microwave assisted extraction (MAE) and enzyme assisted extraction (EAE), can be helpful to improve the extraction efficiency and to reduce environmental impact. Though the execution of these technologies is expensive, they are simply not affordable enough to be employed in any commercial production. Reducing the production cost and increasing the amount of high quality Madhuca longifolia extracts will depend on developing the cost effective and scalable extraction and purification methods.

**LITERATURE REVIEW**

**Ameen et al. (2024)** This study included both authentication and standardization procedures of Physalis angulata through pharmacognostic evaluations combined with phytochemical and chemomicroscopic assessments. The research performed qualitative and quantitative phytochemical screening and used HPLC-DAD methods and GCMS procedures for analysis. Phytochemical screening revealed the existence of flavonoids and terpenes together with alkaloids, tannins and saponins in the tested samples but carbohydrates and glycosides and resins were not detected. HPLC instrument analyzed quercetin, rutin, catechin and ferulic acid whereas GCMS reported findings that included palmitic acid and phytol alongside other bioactive compounds. The pharmacological analysis tested moisture content and total ash and extractive values and showed results which matched WHO standards. The analysis supports the medicinal potential of Physalis angulata which needs additional pharmacology research.

**Asiminicesei et al. (2024)** A study evaluated the effects of heavy metal contamination on medicinal plants' metabolic profiles together with their therapeutic characteristics. The research investigated heavy metal's effects on biosynthetic pathways as it modulates secondary metabolites while negatively impacting medicinal properties of these plants. Antioxidant defenses together with phytohormone signaling appear as essential adaptive mechanisms which plants employ for coping with metal stress. Discussions focused on the possibility to boost plant resistance through genetic alterations of antioxidant-related genetic material. The study introduced the urgency of worldwide joint efforts and progressive protection measures because they both protect medicinal plants from metal pollutants and maintain their therapeutic properties.

**Rajalakshmi et al. (2024)** A study of Mimosa pudica plant morphology was performed to authenticate both the botanical species and maintain its purity. The combination of macroscopical, anatomical, and powder microscopic evaluations showed that Mimosa pudica possesses trichomes, paracytic stomata, starch grains, prismatic crystals, mucilage and brownish contents. A microscopic

quantitative analysis established all measurements of epidermal cells as well as stomatal numbers and index, together with palisade ratios. Bioactive constituents present in ethyl acetate extract contained 13.39 mg/g of quercetin while n-hexane extract contained 27.5 mg/g of β-sitosterol. The experimental results enable differentiation between M. pudica and related plant species or accustomed counterparts.

**Ilangage et al. (2024)** A research study tested medicinal plant extracts against commonly found uropathogenic bacteria using the Brine Shrimp Lethality Assay to determine extract toxicity levels. Antibacterial testing of methanolic extracts focused on the bacterial strains Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The antibacterial action of Phyllanthus emblica fruit extract reached its peak when testing E. coli whereas Boerhavia diffusa root extract demonstrated its best antibacterial response against S. aureus. The root extract of Tragia involucrata inhibited P. aeruginosa to a noteworthy degree. All tested extracts displayed cytotoxic effects through results obtained from Brine Shrimp Lethality Assay (BSLA). This research validates the historical method of using these plants in urinary tract infections while indicating their clinical application potential but demands additional toxicity examinations for safe medicinal use.[75]

**Saidi et al. (2024)** The study evaluated toxic properties of Rhizophora stylosa extracts by implementing the Brine Shrimp Lethality Test (BSLT) to identify their cytotoxic effects. Bark together with leaf and flower extracts were obtained by using three different solvent solutions of n-hexane, dichloromethane, and methanol. The research demonstrated that most test solution extracts were harmless until the dichloromethane extract of bark produced a toxic effect through its LC50 value of 947.76 ppm. Lupeol appeared as the main compound based on the GC-MS examination. The research confirms that traditional R. stylosa extracts show safety characteristics but scientists should analyze the dichloromethane extraction of bark for its future as a potential pharmaceutical compound.

**Wang et al. (2023)** This review demonstrated the critical importance of quality control for herbal medicinal products because it protects both their effectiveness and safety level. Research revealed the growing international demand for herbal treatments as well as the requirement of strict quality control systems which need implementation from development through manufacturing until distribution. The paper specified standardized procedures for contaminant detection together with herbal component authentication and implementation of regulatory protocols. Quality assurance reached its optimal standards through traditional knowledge integration with modern scientific methodology. The review emphasizes that quality control functions as an essential mechanism for developing trust among consumers while shielding public safety while promoting responsible herbal medicine use.

**Chaudhary et al. (2023)** Pharmacognostical standardization of various parts of Euphorbia neriifolia was performed according to WHO protocols. Analysis of quality parameters occurred through three examinations which included morphological assessments together with physicochemical and elemental testing. Total ash and water-soluble ash and acid-insoluble ash concentrations differed between plant parts with latex and leaves having minimum values according to research findings. Only the bark and leaves showed negligible sulfated ash amounts and stems yielded the maximum extractive value (12.794%). Laboratory examinations of this plant detected both primary metabolites including carbohydrates and fixed oils and secondary metabolites including alkaloids and flavonoids and terpenoids and tannins. The research confirmed that heavy metal contents remained inside safe boundaries. Results generated through this research will serve as groundwork to separate and define bioactive compounds from E. neriifolia for pharmacological study purposes.

**Patil et al. (2022)** A scientific investigation of Actiniopteris dichotoma (Mayurshikha) through pharmacognostical and biological evaluation worked to establish its medicinal value. Researchers evaluated A. dichotoma under macroscopic analysis and performed physicochemical and phytochemical assays that confirmed the presence of lipids together with phenols and terpenoids and sterols and alkaloids and flavonoids in the plant specimens. The evaluation of phenolic compounds determined an amount of 6.36 µg/mg GAE. Research testing showed Fraction 4 attained a strong antioxidant capacity through the IC50 value of 1.49 µg/ml and Fractions 3 and 4 prevented microbial growth at 100 µg/ml. The anthelmintic performance along with cytotoxic properties achieved their best results in Fraction 3. Results confirm that A. dichotoma represents an effective medicinal plant which can work as therapeutic solution.

**Pandiyan et al. (2021)** The research evaluated the pharmacological traits and analytical properties of Huberantha senjiana which grows as an endemic species in Gingee Hills districts of Tamil Nadu. Researchers observed single-layered square-shaped epidermal cells under microscopic and macroscopic analysis of the plant tissue together with paracytic stomata and prismatic calcium oxalate crystals and spongy parenchyma and vascular bundles. The powder microscopy analysis verified the presence of fibers and the epidermal trichomes together with xylem vessels. The tests for physicochemical measurements included determination of moisture content and ash value results as well as extractive value measurements and foam index analysis along with fluorescence tests. The phytochemical evaluation detected alkaloids in addition to flavonoids and cardiac glycosides and terpenoids and tannins and steroids. The findings from the elemental testing showed metal amounts inside approved ranges. The reported data serves as indispensable criteria for verifying both the standardization and identification of H. senjiana.

**Moncayo et al. (2021)** The study performed a preliminary phytochemical analysis of western Ecuador native plant species to evaluate their antioxidant activities along with total phenolic and flavonoid components in 18 selected species. Bioactive compounds were identified through the study among various plant species with Adenostemma platyphyllum, Castilla elastica, Ficus brevibracteata, and Passiflora macrophylla. The phytochemical study of Erythroxylum patens achieved 92.41% inhibitory effects when subjected to the DPPH radical scavenging assay assessment. The antioxidant properties of most extracts indicated they could serve as suitable candidates for pharmacological research. The collected data offers significant knowledge for identifying new medicinal natural compounds.

**Suneka et al. (2021)** The Brine Shrimp Lethality Assay (BSLA) established cytotoxicity properties of the extract obtained from Tephrosia purpurea and Andrographis paniculata and Oldenlandia umbellata. Phone technicians extracted dried plant materials using three different processing methods involving cyclohexane extraction through dichloromethane (DCM) combined with

methanol as solvents. Among the obtained extracts using dichloromethane solution demonstrated maximum toxicity effects against the Artemia salina nauplii. The results revealed that the toxicity concentration for DCM extract was 104.712 ppm against T. purpurea, A. paniculata showed 125.89 ppm and O. umbellata achieved 223.872 ppm. Future pharmacological applications will focus on researching bioactive compounds found in dichloromethane extracts of these plants according to scientific studies.

**Shalini et al. (2021)** The research study analyzed phytochemical components as well as the GC-MS spectra and antioxidant quantities present in selected medical plants and their combined herbal remedy PHF. Microscopic, chemical and active chemical properties of Asparagus racemosus, Bauhinia variegata, Caesalpinia bonducella, Saraca asoca, Symplocos racemosa underwent detailed analysis. Research confirmed the existence of alkaloids, flavonoids and carbohydrates, steroids, glycosides, phenols and saponins in the analyzed samples. The results from bioactive compound quantification (TAC, TFC, TGC, TSC, and TPC) were discovered to be positive. The bioactive compounds in the extract were detected through GC-MS analysis. The potent antioxidant properties of PHF indicate possible medical uses for antimicrobial and anti-inflammatory and antiviral and anticancer treatments.

**Zahiruddin et al. (2021)** The research study performed TLC-based metabolite profiling combined with bioactivity validation on water extracts from main Indian medicinal plants which appear in AYUSH formulations. Researchers studied the metabolites found in Phyllanthus emblica together with Piper nigrum, Tinospora cordifolia, Curcuma longa, Ocimum sanctum, Achillea millefolium, Withania somnifera, and Azadirachta indica by utilizing water, ethanol, and hydroethanol solvents. The extracts prepared with water showed optimal antioxidant activities together with superior splenocyte proliferation and pinocytic assay results. A total of 63 and 56 metabolites were detected by TLC analysis when using light wavelengths of 254 nm and 366 nm. Principal component analysis demonstrated different patterns of metabolites through its analysis. Water extraction methods should be employed for polyherbal AYUSH formulations because they enhance the biological effectiveness of such products.

**Zahra et al. (2021)** investigated the antimicrobial, cytotoxic, antioxidant, enzyme inhibition activities, and scanning electron microscopy (SEM) analysis of Lactuca orientalis seeds. Extracts were prepared using six different solvents, with methanol extract (LOSM) showing the highest phenolic (95.76 GAE/mg) and flavonoid (77 QE/mg) content. The LOSM extract exhibited strong antioxidant potential (DPPH scavenging: 82%) and α-amylase inhibition (78.2%), indicating antidiabetic activity. Brine shrimp assay confirmed cytotoxicity, with the highest LD50 for n-hexane extract (13.03 µg/mL). SEM revealed distinct seed surface morphology. The study supports L. orientalis as a promising medicinal plant, warranting further in vivo exploration.

**Nipun et al. (2021)** Research evaluated the pharmacological character of Oldenlandia umbellata to validate its identification protocols along with standardization practices. The scientific analysis proved that Oldenlandia umbellata has strong antibacterial properties along with its anti-inflammatory actions as well as antipyretic effects and hepatoprotective properties and antioxidant capabilities. These analysis methods of stem and leaf examination allowed crucial identification features to become visible in raw drugs. Plant-based research established a complete collection of chemical compositions contained within this particular plant species. Standardization of the crude drug and confirmation of its purity can be achieved through chromo-fingerprinting which analyzes leaves and stems scientifically. These findings help identify O. umbellata and differentiate it from similar Oldenlandia and Hedyotis species to safeguard the crude drug from adulteration.

**Reddy et al. (2021)** Research evaluated the pharmacological aspects of Oldenlandia umbellata to confirm both its identification and standardization methods. The research analysis demonstrated Oldenlandia umbellata possesses potent antibacterial qualities together with anti-inflammatory mechanisms and antipyretic properties and hepatoprotective and antioxidant actions. The stem and leaf microscopic and macroscopic examination showed structural aspects critical for proper identification of crude drugs. The plant-based research determined the diverse list of chemical components found in this particular plant. The scientific method of chromo-fingerprinting analyzed both leaves and stems to achieve standardization and verify purity of the crude drug. O. umbellata identification through these findings enables discrimination from similar Oldenlandia and Hedyotis species thus protecting the crude drug from adulteration.

**Chigurupati et al. (2021)** The study executed a pharmacological investigation of Canna indica leaves extract to measure its protective effects against brain damage and its safety suitability. Special pharmacognostical characteristics emerged from the physicochemical and microscopic analyses. Rats that received methanolic extracts underwent testing for toxicological, antioxidant properties and anatomical structural analysis without noting any behavioral or physical alterations thus indicating safety levels were good. The extract displayed both compatibility with blood cells and an ability to raise reduced glutathione levels in the body. The mechanism of protecting neurons became evident through testing of acetylcholinesterase inhibition at 14.53 µg/mL IC50 which matched Donepezil measurement. Future pharmacological investigation of neuroprotection seems promising due to the findings about C. indica.

**Bijauliya et al. (2021)** A pharmacognostical and physicochemical study on Nyctanthes arbor-tristis leaves was conducted to identify the plant material and create quality control parameters. Results from macroscopical, microscopical, and powder microscopy confirmed all essential diagnostic features in the plant leaves. Testing of phytochemical composition through TLC method was carried out on the crude ethanolic extract during the physicochemical evaluation. The investigation detected alkaloids and glycosides and steroids and phenolics and tannins among the plant constituents. This research helps with N. arbor-tristis standardization which provides accurate methods to identify authentic specimens for medical applications and their potential future therapeutic applications.

**Majee et al. (2021)** This study investigated Trapa natans leaves through pharmacognostical methods for evaluation and identification. The external inspection showed that leaves of Trapa natans appeared in two colors ranging from green to purple while maintaining a rhomboidal shape with toothed margins and pinnate venation pattern. Examination under a microscope revealed that leaves contained a double-sided form which presented an external covering of cuticle and barrel-shaped cells and multiple-trichome structures and stomata patterns of anomocytic type. The assessments and measurements were performed based on WHO guidelines which included physicochemical testing along with extractive value and fluorescence tests. Analysis of plant constituents through phytochemical screening revealed the presence of carbohydrates, alkaloids, glycosides,

**Peter et al. (2020)** Scientists studied the various characteristics of antioxidant properties in addition to pharmacological properties and in silico properties found in Madhuca longifolia leaf extract. The substantial antioxidant properties of Madhuca longifolia leaf extracts became apparent through total phenolic content tests and DPPH assays and measurements of catalase and peroxidase activity in in vitro experiments particularly in aqueous solutions. Testing of a 500 mg/kg dose through pharmacological experiments conducted on female Wistar rats yielded therapeutic outcomes in various models of analgesia and antipyresis and ulcerative disease. Based on researcher simulations M. longifolia compounds and their derivatives including myricetin and quercetin showed strong molecular affinity toward nuclear receptors. M. longifolia leaf has established potential as a drug candidate through this investigation which validates its traditional medicinal practices.

**Jha et al. (2018)** A complete evaluation of Madhuca longifolia was conducted to reveal its phytochemical properties alongside pharmacological effects. The widespread plant encontrado in India, Nepal, and Sri Lanka serves traditional medicine purposes to manage epilepsy and diabetes with its additional functions for inflammation treatment and ulcers management and bronchitis control. Various phytochemical analyses found glycosides with flavonoids along with terpenes and saponins in the plant which enhances its medicinal utility. The plant demonstrates therapeutic effects that include antioxidant properties together with antimicrobial effects and anti-inflammatory powers and anti-diabetic capabilities. The seed oil from this plant serves two functions: biofuel production and human food consumption and its flower blossoms increase male fertility. The authors emphasized future research requirements to examine bioactive compounds and pharmacological applications of the plant.

**Nair et al. (2021)** Scientists established and verified a high-performance thin-layer chromatography analytic technique for the joint assessment of rutin along with quercetin and gallic acid presence in Psidium guajava and Aegle marmelos leaves. The research used toluene:ethyl acetate:formic acid:methanol (3:4:0.8:0.7) as its mobile phase including 254 nm wavelength detection. The precision levels of this method remained under 2% RSD for both intra-day and inter-day measurements as well as inter-analyst analysis. The method achieved detection range between 4.51–5.27 ng/spot while quantification operation stood at 12.73–15.98 ng/spot. The free radical scavenging capability of the substances was proven through the DPPH antioxidant test. The method provides dependable techniques for standardizing polyphenolic compounds in medicinal plants.

## RATIONALE OF THE STUDY

This increasing interest in plant based therapeutics has re-emphasized the need for performing scientific validation of the quality assurance and standardization of the herbal medicines. Large numbers of medicinal plants have been used in traditional medicine systems despite transition into modern pharmaceuticals being scant because of variability of phytochemicals, lack of quality control, lack of validation using the clinical methods and absence of standardized dosage forms. The plant with huge ethnomedicinal importance Madhuca longifolia has been found to have pharmacological potential in the treatment of gastrointestinal, inflammatory conditions, microbial infections, liver diseases and metabolic syndrome. Having been extensively used for traditional applications, the therapeutic potential of this new compound is yet to be well explored by the pharmaceutical industry primarily because of scientific, technical and regulatory constraints. This study is undertaken to bridge the gap between the traditional knowledge and modern medicinally applications of Madhuca longifolia as a medicinal herb with regard to establishment of a complete quality audit framework, evaluation and standardization approaches and pharmacological validation of the herb.One of the crucial problems in development of herbal drugs is a lack of chemical uniformity and reproducibility of phytochemical composition. Madhuca longifolia, a medicinal plants, presents variability of phytochemicals because of the environmental, geographical, seasonal and agronomic factors that influence biosynthesis of secondary metabolites. Flavonoids, saponins, tannins, alkaloids and terpenoids concentrations are high hence there is no guarantee of uniformity of preparations hence batch to batch concentration varies depending on the composition of the soil, climatic condition on the growth growth stage and in extraction processes. Weather this affects the efficacy and safety of Madhuca longifolia derived medicinal products in a therapeutic way and is responsible for the inconsistency and, consequently, standardization becomes a prerequisite for such applications. The overall objective of this study is to obtain comprehensive phytochemical profiling utilizing chromatographic and spectroscopic techniques for the identification and quantitation of the bioactive constituents in order to establish quality control standards for the metabolites. Phytochemical fingerprints will facilitate consistency in medicinal properties and uniform therapeutic effects between pharmaceuticals, and between the two.

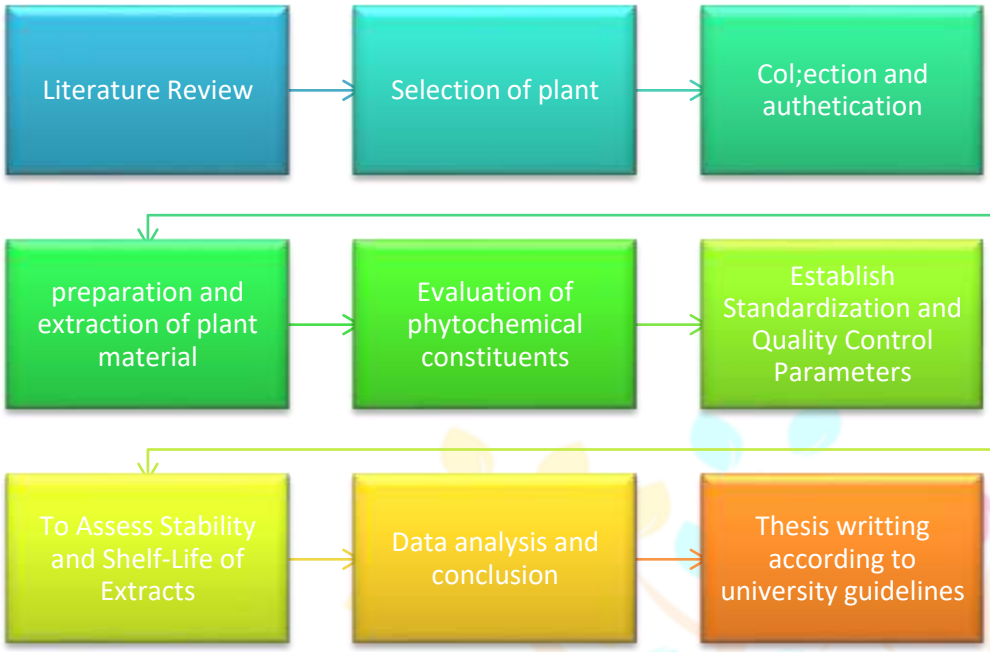
## AIM

To perform Quality Audit of Madhuca Longifolia as a Medical Harb

## OBJECTIVES

1. To Collect and Authenticate Madhuca longifolia Samples.
2. To prepare and extract plant material using appropriate method and solvent.
3. To Evaluate the Phytochemical Composition.
4. To Establish Standardization and Quality Control Parameters.
5. To Assess Stability and Shelf-Life of Extracts.
6. To Optimize Extraction Techniques for Maximum Bioactive Yield.
7. To Develop a Comprehensive Quality Assurance Framework for Madhuca longifolia

**PLAN OF WORK**




**PLANT PROFILE**

| **Madhuca longifolia** | |
|---|---|
|  | |
| **Scientific Name** | Madhuca longifolia (J. Koenig ex L.) J. F. Macbr. |
| **Family** | Sapotaceae |
| **Common Names** | Mahua, Butter Tree, Illuppai, Indian Butter Tree, Moha Tree, Madhuka |
| **Taxonomy** | **Kingdom** :Plantae<br>**Phylum** : Tracheophyta<br>**Class** ;Magnoliopsida<br>**Order** ;Ericales<br>**Family** :Sapotaceae<br>**Genus** :Madhuca<br>**Species** :Madhuca longifolia |
| **Plant Type** | Medium to large-sized deciduous tree, growing up to 20 meters in height, with a deep-rooted system, wide-spreading canopy, and drought-resistant properties. |
| **Origin and Distribution** | Native to India and widely found in South Asia, including Sri Lanka, Nepal, Bangladesh, Myanmar, and Malaysia, with scattered populations in parts of Africa and Southeast Asia. |
| **Morphology** | Thick, dark-colored, deeply fissured bark exuding milky latex; simple, alternate, oblong leaves (10-20 cm long); small, yellowish-white, fragrant flowers in dense clusters; fleshy ellipsoid fruits turning yellow-brown when ripe; oil-rich brown seeds. |
| **Cultivation** | Thrives in tropical and subtropical climates, prefers well-drained sandy or loamy soil; propagated through seeds and vegetative methods; drought-resistant and moderately salt-tolerant. |
| **Flowering and Fruiting Season** | Flowers bloom between February and April, attracting pollinators like bees and bats; fruiting occurs from May to July, with seeds harvested between June and August. |
| **Economic Importance** | Flowers used in alcohol production, natural sweeteners, and animal fodder; seeds processed for oil extraction; bark, leaves, and seeds used in herbal medicine; mahua oil used in pharmaceuticals, cosmetics, and biodiesel production. |
| **Traditional Medicinal Uses** | Used in Ayurveda, Siddha, and folk medicine for ulcers, inflammation, diabetes, wounds, skin infections, fever, bronchitis, gastrointestinal disorders, and rheumatism. |
| **Phytochemical Constituents** | Rich in flavonoids (quercetin, kaempferol, luteolin), saponins, tannins, alkaloids, |

| | |
|---|---|
| | steroids, phenolic acids (gallic acid, caffeic acid), glycosides, terpenoids, and triterpenes. |
| **Recent Scientific Studies** | Confirmed anti-inflammatory, hepatoprotective, antimicrobial, antioxidant, and wound-healing activities; potential roles in diabetes management, neuroprotection, and anti-cancer applications. |
| **Potential Therapeutic Uses** | Traditionally and scientifically studied for ulcers, liver disorders, skin diseases, wounds, metabolic syndromes, and oxidative stress; potential for immunomodulatory, analgesic, and cardiovascular protection. |
| **Toxicity and Safety Concerns** | Generally safe, but excessive consumption of fermented flower products may cause intoxication; high doses of seed oil can lead to gastrointestinal discomfort. |
| **Regulatory Status** | Recognized under AYUSH in India and included in the Ayurvedic Pharmacopoeia; requires compliance with WHO, USP, EMA, and ICH guidelines for global herbal medicine regulation. |
| **Industrial Applications** | Used in pharmaceuticals, nutraceuticals, cosmetics, and food industries; mahua oil utilized in skincare for anti-aging and wound-healing; key role in biodiesel and eco-friendly products. |
| **Sustainability and Conservation Status** | Though widely cultivated, overharvesting for commercial use raises conservation concerns; sustainable agroforestry and controlled harvesting practices are encouraged. |

## MATERIALS AND METHODS

### Materials

**Table 6.1: List of materials used for research work**

| Sr. No. | Chemical/Reagent | Purpose | Source |
|---|---|---|---|
| 1 | Ethanol (70%) | Solvent for extraction | Merck India, Mumbai |
| 2 | Petroleum Ether | Solvent for non-polar compound extraction | Loba Chemie, Mumbai |
| 3 | Chloroform | Solvent for alkaloid extraction | HiMedia, Mumbai |
| 4 | Methanol | Solvent for fractionation & chromatography | Research-Lab Fine Chem, Pune |
| 5 | Ethyl Acetate | Solvent for flavonoid separation | Sisco Research Laboratories, Mumbai |
| 6 | Formic Acid | Reagent for TLC solvent system | Rankem, Maharashtra |
| 7 | Acetic Acid | Reagent for TLC solvent system | Loba Chemie, Mumbai |
| 8 | Ammonia Solution | Reagent for alkaloid detection | Thermo Fisher Scientific, Mumbai |
| 9 | Silica Gel (60-120 mesh) | Stationary phase for column chromatography | Sigma-Aldrich, Mumbai |
| 10 | Dragendorff's Reagent | Alkaloid detection reagent | Loba Chemie, Maharashtra |
| 11 | Mayer's Reagent | Alkaloid detection reagent | HiMedia, Maharashtra |
| 12 | Ferric Chloride | Phenolic compound detection | SDFCL, Maharashtra |
| 13 | Lead Acetate | Tannin detection reagent | Central Drug House (CDH), Mumbai |
| 14 | Brine Shrimp Eggs | Cytotoxicity testing (Brine Shrimp Lethality Assay) | Local Aquarium Supply, Maharashtra |
| 15 | Distilled Water | General laboratory reagent | Lab-prepared, Pune |

**Table 6.2: List of Instruments/Equipments used for research work**

| Sr. No. | Instrument/Equipment | Purpose | Source |
|---|---|---|---|
| 1 | Rotary Evaporator | Solvent evaporation under reduced pressure | Buchi India Pvt. Ltd., Mumbai |
| 2 | UV-Visible Spectrophotometer | Compound analysis and quantification | Shimadzu, Mumbai |
| 3 | High-Performance Liquid Chromatography (HPLC) | Compound separation and identification | Agilent Technologies, Pune |
| 4 | Fourier Transform Infrared Spectroscopy (FTIR) | Functional group analysis of compounds | Bruker, Mumbai |
| 5 | Thin Layer Chromatography (TLC) Chamber | Separation and identification of phytochemicals | Local Glassware Supplier, Pune |
| 6 | Digital Weighing Balance | Accurate measurement of sample weight | Sartorius, Mumbai |
| 7 | Hot Air Oven | Drying and sterilization of samples | Thermo Fisher Scientific, Mumbai |
| 8 | pH Meter | Determination of pH of extracts | Eutech Instruments, Mumbai |
| 9 | Sonicator | Extraction and dissolution of compounds | PCI Analytics, Mumbai |
| 10 | Soxhlet Apparatus | Continuous extraction of plant material | Borosil, Maharashtra |

| | | | |
|---|---|---|---|
| 11 | Column Chromatography Setup | Fractionation and purification of extracts | Local Lab Supplier, Pune |
| 12 | Microscope | Microscopic examination of powdered drug | Olympus India, Mumbai |
| 13 | Incubator | Microbial testing and culture incubation | Remi Lab Instruments, Mumbai |
| 14 | Water Bath | Heating samples at controlled temperatures | PCI Analytics, Maharashtra |
| 15 | Centrifuge Machine | Separation of sample components by density | Remi Elektrotechnik, Mumbai |

**Methodology**

**Collection and authentication of plant**

The plant of Madhuca longifolia was carefully collected from the local region of Chhatrapati Sambhajinagar (Aurangabad) District, Maharashtra, India, ensuring that it was obtained in its optimal condition for scientific evaluation. Following collection, a herbarium specimen was meticulously prepared using standard botanical preservation techniques to maintain its morphological characteristics for future reference. The prepared herbarium specimen was then submitted to The pharma research companion lab, Pune, Maharashtra. After thorough verification and authentication by experts, the plant was assigned the unique reference number 2025/PRC/DD/0049 for precise identification and traceability. The authenticated herbarium specimen has been securely archived for future reference, serving as a documented record for any subsequent scientific investigations or taxonomic validation of Madhuca longifolia.

**6.2.2. Preparation of Collected plant material**

Tactfully, the plant of Madhuca longifolia was cleaned by tap water to get rid of the dust, dirt, and the stuck foreign particles. Finally, the cleaned plant material was shade dried at room temperature for 15–20 days to retain its phytoconstituents but with low moisture content. During drying, a regular monitoring was carried out in order not to be contaminated by microbes or fungal growth. The plant material was completely dried and then coarsely powder using a mechanical grinder and passed through a 40 mesh sieve to obtain a uniform particle size. The finely powdered material was then placed in an airtight container under controlled storage conditions in order to prevent exposure to light, moisture, and environmental conditions. Subsequently, the prepared plant powder was used for carrying out various analytical and experimental projects.

**Figure 6.1: Prepared plant material**

**Extraction of plant material**

For the isolation of both the non Polar and the polar phytoconstituents of the dried and powdered plant material of Madhuca longifolia, sequential petroleum ether (40–60°C) and hydroalcoholic solvent (ethanol: water 70:30) extraction was done. A Soxhlet apparatus was used to initially extract 500 g of plant powder with petroleum ether until the siphon became colorless, then the extract was filtered and its concentration was done using a rotary evaporator at 40°C under reduced pressure and stored in an amber color bottle at 4°C for further analysis. The second time, another 500 g of plant material were macerated in 70% ethanol (1:10 w/v) at room temperature for 48–72 hours with shaking and then filtered through Whatman No. 1 filter paper. The concentrate from the filtrate was concentrated using rotary evaporator at 45 °C under reduced pressure and yielded a semi solid extract that was further dried in vacuum desiccator and stored at 4 °C for phytochemical and pharmacological investigations. Following the equation, the yield percentage was used to calculate the extraction efficiency, then the obtained extracts were subjected to further process.

$$\% \text{ Yield} = \frac{\text{Weight of the dried extract}}{\text{Weight of the initial plant material used}} \times 100$$

**Figure 6.2: Extraction of plant material by soxhlet extraction**

**Preliminary phytochemical analysis**

**A. Alkaloid Tests**

**Dragendorff's Test**

2 mL of the plant extract was carefully mixed with 1 mL of Dragendorff's reagent, which is a solution of potassium iodide and bismuth nitrate. The mixture was allowed to react for a few minutes, and the formation of an orange or reddish-brown precipitate was observed, indicating the presence of alkaloids.

**Mayer's Test**

2 mL of the plant extract was treated with 1 mL of Mayer's reagent, which consists of potassium mercuric iodide. The test tube was gently shaken to ensure proper mixing, and the appearance of a cream-colored precipitate confirmed the presence of alkaloids.

**Hager's Test**

A further confirmation of alkaloids was performed using Hager's reagent, a saturated picric acid solution. In this test, 2 mL of the extract was combined with 1 mL of Hager's reagent. The development of a yellow precipitate indicated the presence of alkaloids in the plant sample.[98,99]

**Wagner's Test**

To verify alkaloids, 2 mL of the extract was treated with 1 mL of Wagner's reagent, which contains iodine in potassium iodide solution. The mixture was observed for the formation of a reddish-brown precipitate, which confirmed the presence of alkaloids in the extract.

**B. Carbohydrate Tests**

**Molisch's Test**

To test for the presence of carbohydrates, 2 mL of the extract was mixed with 2 drops of Molisch's reagent (a solution of α-naphthol in ethanol). This was followed by the slow addition of 1 mL of concentrated sulfuric acid along the inner wall of the test tube. A purple or violet ring formed at the interface between the two layers, confirming the presence of carbohydrates in the extract.

**Fehling's Test**

For detecting reducing sugars, 1 mL each of Fehling's A (copper sulfate solution) and Fehling's B (potassium tartrate and sodium hydroxide solution) were mixed and added to 2 mL of the plant extract. The test tube was heated in a boiling water bath for 5 minutes, and the formation of a brick-red precipitate confirmed the presence of reducing sugars in the extract.

**C. Glycoside Tests**

**Keller-Killiani Test**

To test for cardiac glycosides, 2 mL of the extract was treated with 1 mL of glacial acetic acid, followed by the addition of one drop of ferric chloride solution. Then, 1 mL of concentrated sulfuric acid was carefully added along the inner wall of the test tube without disturbing the layers. The appearance of a brown ring at the interface between the liquids confirmed the presence of cardiac glycosides in the plant extract.

**Borntrager's Test**

For the detection of anthraquinone glycosides, 2 mL of the extract was mixed with 2 mL of chloroform and vigorously shaken. The chloroform layer was separated, and 1 mL of dilute ammonia solution was added to it. The development of a pink, red, or violet color in the ammonia layer indicated the presence of anthraquinone glycosides in the plant material.

**D. Steroid Tests**

**Libermann-Burchard Test**

To identify steroids, 2 mL of the extract was treated with 2 mL of acetic anhydride and mixed well. Then, 1 mL of concentrated sulfuric acid was carefully added along the test tube wall. A greenish-blue or bluish-green color that appeared in the solution confirmed the presence of steroids.

**Salkowski Test**

Another test for steroids involved adding 2 mL of the extract to 2 mL of chloroform and mixing well. Then, 2 mL of concentrated sulfuric acid was carefully introduced along the inner wall of the test tube. The formation of a red coloration in the chloroform layer and a yellow-green fluorescence in the acid layer indicated the presence of steroids in the extract.

**E. Flavonoid Tests**

**Shinoda's Test**

To confirm the presence of flavonoids, 2 mL of the extract was treated with few magnesium turnings, followed by the slow addition of 1 mL of concentrated hydrochloric acid. A pink, red, or orange coloration developed in the solution, confirming the presence of flavonoids in the extract.

**Lead Acetate Test for Flavonoids**

A further confirmation of flavonoids was carried out by adding 1 mL of lead acetate solution to 2 mL of the extract. The appearance of a yellow precipitate confirmed the presence of flavonoids.

**F. Saponin Test**

**Foam Test**

To detect saponins, 2 mL of the extract was vigorously shaken with 5 mL of distilled water in a test tube. The formation of a stable frothy foam, which persisted for at least 10 minutes, indicated the presence of saponins in the extract.

**G. Tannin Test**

**Lead Acetate Test for Tannins**

To confirm the presence of tannins, 2 mL of the extract was mixed with 1 mL of lead acetate solution. The development of a white or yellow precipitate in the solution confirmed the presence of tannins in the extract.

**H. Phenol Test**

**Ferric Chloride Test**

For the detection of phenolic compounds, 2 mL of the extract was treated with 3–4 drops of ferric chloride solution. The formation of a blue-green or black coloration indicated the presence of phenols in the extract.[100–102]

**Figure 6.3: Preliminary phytochemical investigation**

**6.2.5. Quantitative Phytochemical Analysis of Madhuca longifolia**

**A. Total Alkaloid Content**

A known weight of the plant extract (5 g) was dissolved in 200 mL of 10% acetic acid in ethanol in a conical flask to determine the actual total quantity of alkaloids in the extract. Stirred thoroughly the mixture and allowed them to stand undisturbed for 4 hour at room temperature to sure extraction of alkaloids in the acidic medium. The incubated solution was filtered through Whatman No. 1 filter paper to remove any plant residues and the filtered through filtrate was then carefully concentrated in a water bath at a controlled temperature to avoid degradation of the alkaloid compounds. About one fourth of the volume was reduced and ammonium hydroxide solution was added drop by drop with continuous stirring until precipitation of alkaloids is complete. Filtration, washing with double distilled water, drying in a hot air oven at 60°C and weighing of the precipitate. The amount of alkaloids was calculated by formula:

$$\text{Total Alkalod Content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

**Figure 6.4: Total alkaloid content determination**

**B. Total Flavonoid Content**

It was estimated that total flavonoid content would be measured by the specific and sensitive method for flavonoid compounds - Aluminum Chloride Colorimetric Method. With say a 1 ml aliquot of the plant extract, a 4 ml of distilled water was added in a test tube and the two are mixed together. To this mixture, 0.3 mL of a 5% sodium nitrite ($NaNO_2$) solution was added, we beat the mixture for 5 minute. Thus, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added to the reaction mixture and stood for another 5 minutes to trap the flavonoids into aluminum chloride. Afterward, 1M sodium hydroxide (NaOH) solution was added to the solution to make it alkaline and the final volume was brought to 10 mL with distilled water. Absorbance was measured at 510 nm with UV Visible Spectrophotometer, and mixed solution was thoroughly mixed and used to investigate. The flavonoid content was done using a quercetin standard curve with results reported as mg quercetin equivalent (QE) per gram extract.

**C. Total Phenolic Content**

The content of phenolic was quantified in plant extract through Folin Ciocalteu based on the oxidation of phenols in alkaline solution. Finally, 2.5 mL of 10% Folin-Ciocalteu reagent was added to a 1 mL aliquot of the plant extract and incubated at room temperature after mixing for 5 minutes to get the first oxidation reaction. After this time, 2.5 mL of 7.5% sodium carbonate ($Na_2CO_3$) solution was added and the reaction mixture was incubated for 30 min in the dark to avoid any interference of light. A UV-Visible spectrophotometer was used to record the absorbance of the blue colored complex formed and also against a blank. The values reported represent the amount of gallic acid equivalent (GAE)/g of extract and were obtained from a gallic acid standard curve for the total phenolic content.

**Figure 6.5: Total phenolic content determination**

**D. Total Tannin Content**

The Folin-Denis method was used to estimate the total tannin content which is specific for tannins by producing a blue colored complex with them. Therefore, the mixture of 1 mL of the plant extract and 7.5 mL of distilled water with 0.5 mL of Folin-Denis reagent (phosphomolybdotungstic acid solution) that reacts with tannins. In order to improve the reaction, 1mL of 10% sodium carbonate ($Na_2CO_3$) solution was added to a reaction mixture and its volume was set to 10mL. The mixture was also incubated for 30 minutes at room temperature at 37°C to allow complete color development. Solution was measured for the absorbance at 700 nm in a UV-Visible spectrophotometer. A tannic acid standard curve was used to calculate the total tannin content which was reported as mg of Tannic Acid equivalent of extract of berries per gram of extract.

**E. Total Saponin Content**

The gravimetric method was hence used to determine the total saponin content. A 20% ethanol solution with a 50 mL volume was used to dissolve a 5 g sample of the plant extract and perform complete extraction of saponins at a temperature of 55°C in a water bath with constant stirring for 4 hours. The residue was re-extracted with an additional 50 mL of 20% ethanol, to which the mixture was then filtered. The filtrates were concentrated to 20 mL and transferred to a separating funnel, and 20 mL of diethyl ether were added to remove any unwanted non saponin impurities. The aqueous layer was collected after which 5 mL of n butanol was added to improve saponin extraction. The saponin fraction was evaporated to dryness in an oven at 60°C and the saponin fraction was weighed as dried material. The values for the content of this saponin were calculated.

$$\text{Total Saponin Content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$


**Figure 6.6: Total saponin content determination**

**F. Total Glycoside Content**

In this way, the total glycoside content has been calculated by Baljet's method through colorimetric estimation. Two milliliters of freshly prepared Baljet's reagent were added to a 2 mL aliquot of the plant extract and mixed; the mixture was allowed to stand

for 1 hour at room temperature with no disturbance for color development. The mixture was incubated, then 1 mL of glacial acetic acid was added to make acidic, and the absorbance at 495 nm was measured using a UV-Visible spectrophotometer. The contribution of glycosides to the total content was calculated according to a digoxin standard curve and was expressed as mg digoxin DE/g extract.

**Figure 6.7: Total glycoside content determination**

**G. Reducing Sugar Content**

The reducing sugars were measured using the DNSA method (3,5-Dinitrosalicylic Acid) by reacting reducing sugars with a colored complex which is formed. Extract (1 mL) was mixed with 3 mL of DNSA reagent and boiled in a boiling water bath 5 min. The reaction was stopped by cooling down the mixture under running tap water after heating the mixture. The reducing sugar content was determined by increasing the absorbance of the solution at 540 nm and then using a glucose standard curve.

**Figure 6.8: Total reducing sugar content determination**

**H. Total Protein Content**

Lowry's method was used for the estimation of the total protein content that is based on reaction between the protein molecules and Folin's reagent that forms a blue colored complex. A volume of 1 mL of the extract was mixed with 5 mL of Lowry's reagent and incubated for 15 minutes to allow protein complex formation with copper. Folin's reagent was added to the reaction mixture after this, as well as 0.5 mL of Folin's reagent, which left the mixture incubated for an additional 30 minutes in order to develop color. The absorbance at 660 nm was measured of the obtained solution using a UV-Visible spectrophotometer and the protein content was expressed as mg bovine serum albumin (BSA)/g of extract using a standard calibration curve.

**Determination of Extractive values**

**A. Alcohol-Soluble Extractive Value**

Searched Dried Madhuca longifolia powder, about 5 g was placed in to a conical flask and then 100 mL of ethanol (95%) was added. It was tightly sealed in the flask and stored at room temperature for 24 hrs including slight shaking to perform extraction of alcohol soluble substances like alkaloids, glycosides, flavonoids, tannins and phenolics. After extraction period, the mixture was filtered using Whatman No. 1 filter paper and 25ml of the filtrate was allowed to evaporate to dryness in pre weighed evaporating dish using water bath at 60°c. The dish was then dried in a hot air oven at 105°C, at cooled in desiccator and weighed. The formula was used to calculate the percentage of alcohol soluble extractive value.

$$\text{Alcohol} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**Figure 6.9: Alcohol soluble extractive value determination**

**B. Water-Soluble Extractive Value**

A 250 mL conical flask was charged with 100 mL distilled water and then about 5 g of dried Madhuca longifolia powder was placed in the cone. A solution for extraction of the water soluble constituents of saponins, carbohydrates, tannins, mucilage, gums and proteins was made by sealing the flask and shaking it frequently for 24hrs at room temperature to aid in extraction. Twenty-five millilitre from the clear filtrate was taken and the extract was evaporated to dryness to a pre weighed evaporating dish in a water bath at 60 C. The dish was then dried in a hotair oven at 105°C, and cooled in a desiccator and then weighed. Based on the formula this percentage of the water soluble extractive value was calculated.

$$\text{Water} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**C. Petroleum Ether-Soluble Extractive Value**

A 250 mL of conical flask was charged with 100 mL of petroleum ether (40-60°C boiling range) and to it, 5 g of dried powder of Madhuca longifolia was placed. The flask was tightened and subsequently left to stand for 24 hr at room temperature with occasional shaking to extract non polar constituents like fixed oils, fats, steroids and lipophilic compounds. After removal of the extract period, the extract was filtered through Whatman No. 1 filter paper and 25 mL of filtrate was evaporated to dryness in a preweighed evaporating dish using a water bath at 60°C. Following, the dish was then air dried in a hot air oven at 105°C, then weighed in a desiccator. Total value of petroleum ether soluble extractive for same was derived by using the formula.

$$\text{Petroleum ether} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**D. Chloroform-Soluble Extractive Value**

For the experiment, a 5 g sample of dried powder of Madhuca longifolia was stirred into a 250 mL conical flask with 100 ml of chloroform. The flask was sealed, stood at room temperature for 24 hours and occasionally shaken to extract semipolar (alkaloids, flavonoids and terpenoids) constituents. The filtrate was then filtered using Whatman No. 1 filter paper and 25 mL of the filtrate appeared dry in a pre-weighed evaporating dish using a water bath and heated to 60°C. In the hot air oven at 105°C the dish was further dried, cooled in desiccator and weighed. The formula for calculating the chloroform soluble extractive value was:

$$\text{Chloroform} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**E. Ether-Soluble Extractive Value**

For 5 g of dried Madhuca longifolia powder, a 250 mL conical flask is used, and 100 mL of diethyl ether is added to the sample. As they extract volatile compounds, essential oils, lipophilic constituents, flask was tightly sealed and left to stand for 24 hours at room temperature in between shaking. The extract was filtered through Whatman No. 1 filter paper to which 25.0 mL of filtrate was added and evaporated to dryness on a pre weighed evaporating dish by means of a water bath at 60°C. The residue was further dried in a hot air oven at 105°C, cooled in desiccator and weighed. The formula was used for calculation of the ether soluble extractive value.[115]

$$\text{Ether} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**Figure 7.10: Ether soluble extractive value determination**

**F. Acetone-Soluble Extractive Value**

In a 250 mL conical flask, a 100 mL acetone was added into a 5 g sample of dried Madhuca longifolia powder. Semi polar constituents such as flavonoids, glycosides, terpenoids etc were extracted from the flask which was sealed and kept at room temperature and at shaking intervals for 24 hours. The clear filtrate [from the extraction period] was filtered through Whatman No. 1 filter paper, and 25 mL of it was evaporated to dryness in a preweighed evaporating dish in a water bath hair 60°C. The dish was hot air oven dried at 105°C, desiccated, then weighed. The following formula was used to determine acetone solublo extractive value.

$$\text{Acetone} - \text{soluble extractive value (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

**6.2.7. Ash Value Determination**

**A. Total Ash Value**

To estimate total inorganic content in the plant material (physiological and non physiological ash), the total ash value was found. A pre weighed silica crucible was used to accurately weigh 2 g of dried powdered Madhuca longifolia and incinerated in a muffle furnace to 500–600°C until the powder became white carbon free ash. After that, the crucible was cooled in a desiccator and the final weight of the ash recorded. The formula was used to calculate the total ash value.

$$\text{Total ash value (\%)} = \frac{\text{weight of ash}}{\text{weight of sample taken}} \times 100$$

**B. Acid-Insoluble Ash Value**

The amount of siliceous matter in the plant material, such as sand and silica, was determined by means of the acid insoluble ash value. The previous step resulted in the total ash being treated with 25 mL of dilute hydrochloric acid (HCl, 2N) and gently boiled for 5 minutes. The residue of the mixture was washed until acid free with hot distilled water which was then filtered through ashless filter paper (Whatman No. 41). It was subsequently transferred back to the preweighed silica crucible, ignited in a muffle furnace at 500–600°C until the residue was completely white. The crucible was weighed, cooled in a desiccator, the ash calculated and the acid insoluble ash as used the formula.[118]

$$\text{Acid insoluble ash value (\%)} = \frac{\text{weight of acid insoluble ash}}{\text{weight of sample taken}} \times 100$$

**C. Water-Soluble Ash Value**

The total ash was quantified to determine the proportion of total ash which is water soluble (i.e. solubility in water) indicating the presence of inorganic compounds, such as salts and minerals, and is commonly expressed as ash on anomalous free carbon (AFC). The previously obtained total ash was boiled for 5 min with 25 mL of distilled water. The mixture was applied to ashless filter paper (Whatman No. 41) and the insoluble residue was removed and transferred into the pre weighed silica crucible. It was then ignited in a muffle furnace at 500–600°C until a constant weight is obtained. Weight of the total ash water insoluble residue was calculated by forming its difference to identify the water soluble ash residue and then formula was used to find the amount.

$$\text{Water soluble ash value (\%)} = \frac{\text{Weight of total ash} - \text{Weight of water insoluble residue}}{\text{weight of sample taken}} \times 100$$

**Figure 6.11: Water soluble extractive value determination**

**D. Sulphated Ash Value**

Where bound water or volatiles were present, the Toluene Distillation Method was also used as a substitute for the moisture determination. For determination of the moisture of a 5 g sample of Madhuca longifolia powder, 100 mL of toluene is added to Moisture Determination Apparatus. The mixture was then gently heated in a water bath until distilled moisture was collected in a

graduated tube for 3 to 4 hours. The receiver collected water and was measured by the volume collected and the moisture expressed as:

$$\text{Sulphated ash value } (\%) = \frac{\text{Weight of sulphated ash}}{\text{weight of sample taken}} \times 100$$

**Moisture Content Determination**

**A. Loss on Drying (LOD) Method**

The moisture content of Madhuca longifolia was determined using the Loss on Drying (LOD) method, which measures the amount of water and volatile substances present in the plant material. A 5 g accurately weighed sample of the powdered plant material was placed in a pre-weighed porcelain or glass crucible and dried in a hot-air oven at 105°C for 4–6 hours until a constant weight was achieved. The sample was then cooled in a desiccator and weighed again. The percentage of moisture content was calculated using the formula:

$$\text{Moisture content } (\%) = \frac{\text{Initial weight of sample} - \text{Final weight after drying}}{\text{Initial weight of sample}} \times 100$$

**Figure 6.12: loss on drying by hot air oven**

**B. Toluene Distillation Method**

Where bound water or volatiles were present, the Toluene Distillation Method was also used as a substitute for the moisture determination. For determination of the moisture of a 5 g sample of Madhuca longifolia powder, 100 mL of toluene is added to Moisture Determination Apparatus. The mixture was then gently heated in a water bath until distilled moisture was collected in a graduated tube for 3 to 4 hours. The receiver collected water and was measured by the volume collected and the moisture expressed as:

$$\text{Moisture content } (\%) = \frac{\text{Volume of water collected (mL)}}{\text{Weight of sample}} \times 100$$

**Pharmacognostical Evaluation of Madhuca longifolia**

**A. Macroscopic Analysis**

The collection, armed with appropriate tools, of different parts of Madhuca longifolia, leaves, bark, flowers, fruit and seeds were made to evaluate them macroscopically to document their general color, texture, shape and size. Magnifying lenses and calipers were used to observe the samples on a white background and make measurements with a level of precision. Touch and feel were done to the texture and sensory evaluation methods were used for odor and taste. To compare standard monographs it was described in a detailed morphological description.

**B. Microscopic Analysis**

Thin transverse sections (T.S.) of fresh and dried samples of Madhuca longifolia leaves, bark and seed were obtained by the use of a sharp razor blade and were placed onto clean glass slides for microscopic evaluation. First those sections were observed under a compound microscope in normal light. Sections were stained with safranin and fast green and the visualization of cellular structures was improved. Air bubbles were prevented by mounting the prepared slides with glycerin and covering with a cover slip. Study of epidermal cells, stomatal patterns, vascular bundles and trichomes was done in the slides which were examined under a compound microscope at different magnifications viz. (10x and 40x). The systematic observations were recorded and documentation of the photo micrographs were taken.

**Figure 6.13: Microscopic analysis**

**Qualitative Heavy Metals Testing**

Simple qualitative tests were thus performed to carry out preliminary heavy metal detection in Madhuca longifolia. Aqueous extract of the plant material was prepared from 2 g of sample by boiling in 10 mL of distilled water, filtered and 2 to 3 drops of 10% sodium sulfide ($Na_2S$) solution were added and in the presence of Pb, a black or brown precipitate was formed. Plant extract (2 mL) was treated with potassium cyanide (KCN) solution and dilute hydrochloric acid (HCl) was added to the mixture, a yellow precipitate indicated the formation of cadmium. It performed arsenic (As) detection using the Gutzeit test in which 5mL of zinc (Zn) granules and dilute HCl is added to 2mL of plant extract, and a filter paper soaked in silver nitrate solution ($AgNO_3$) solution is pasted over the test tube; if there is arsenic contamination then yellow as well brown stain appears on the paper. Diphenylcarbazone test was used for the detection of mercury (Hg) where 2 mL plant extract was treated with 2–3 drop of diphenylcarbazone reagent, making formation of violet or purple colour that prone mercury ion. The preliminary qualitative tests performed served as a rapid screening of heavy metals in plant material.

**TLC (Thin Layer Chromatography)**

Specific bioactive compounds of Madhuca longifolia hydroalcoholic extract were isolated by various solvents having different polarity for separation of various phytoconstituents during analytic TLC. The choice of solvent systems was made to target distinct classes of compounds: Toluene : Ethyl Acetate : Formic Acid (5:4:1) was selected for its efficiency in separating moderately polar constituents; Chloroform : Methanol : Ammonia (8:2:0.5) was chosen for its ability to facilitate the migration of basic alkaloidal components; Ethyl Acetate : Formic Acid : Acetic Acid : Water (10:1.1:1.1:2.6) was used to enhance separation of polar phytochemicals such as glycosides and phenolics; and Toluene : Chloroform : Methanol (5:4:1) was employed for isolating non-polar to semi-polar constituents such as steroids and terpenoids.

Pre coated silica gel 60 $F_{254}$ plates (20 cm × 20 cm, 1 mm thickness) were used for preparative TLC, and activated at 110°C for 30 minutes. A 500 mg extract in 5 mL solvent (one solute) solution concentration was made, and a 2 cm wide band was applied as a base on the TLC plate. The plates were developed up to 15 cm with the solvent front using the respective mobile phase, and the visualisation of the bands was done by UV (254 nm and 366 nm) and detection by use of appropriate spray reagents. Target compounds were carefully scraped off with a glass spatula, transferred to proper solvents, methanol, chloroform, ethanol or petroleum ether, passed through Whatman No. 1 filter paper, finally concentrated under reduced pressure at 40–50°C using rotary evaporator. The reconstituted fractions were further characterized by means of UV-Visible spectrophotometric analysis.

**Figure 6.14: Preparative TLC for scrapping compound**.

**Standardization and Quality Assurance**

After standardization and quality assurance of Madhuca longifolia under WHO, AYUSH and pharmacopoeial guidelines, it was established the identity, purity, safety and consistency. Natural source of the plant material was botanically authenticated, finally physicochemical evaluation of the plant material was carried out for moisture content, total ash, acid removable ash, water soluble ash and extractive values to determine purity and chemical composition. Qualitative screening, TLC and HPLC were used to develop a fingerprint profile and identify the major bioactive constitutents in the phytochemical profiling. Further, the extract was used to fractionate by column chromatography and representative isolated compounds were characterized by UV-Visible Spectrophotometry, and FTIR, and NMR analytes and confirmed by mass spectroscopy in the Positive Electrospray Tandem Mass Spectrometry (ESI-MS) mode. The microbial load was tested by analyzing for the presence of bacterial and fungal contaminants. This includes Escherichia coli, Salmonella spp., Staphylococcus aureus and Pseudomonas aeruginosa, and heavy metals were checked for absence of toxic elements, lead, cadmium, arsenic and mercury. As part of its safe profile, cytotoxicity was evaluated using the brine shrimp lethality assay. All analytical findings were compared with the pharmacopoeial standards for herbal drug quality assurance in compliance to the regulatory guidelines.

**RESULT AND DISCUSSION**

**Extraction Yields**

Madhuca longifolia extract yield obtained differed greatly by polarity of solvent, hydroalcoholic (70%) extract having 14.8 ± 0.9% and petroleum ether extract 4.2 ± 0.3%. This definitely highlights a typical excess of polar and semi polar phytoconstituents like flavonoids, phenolics and saponins in plant matrix and are generally better dissolved in ethanol – water mixtures. However, the lower yield by the use of petroleum ether is due to their preference for non polar compounds like lipids, steroids and terpenoids, which are present in relatively less quantity. The result is consistent with previous pharmacognosticial studies that characterize the solvent dependent bioactive content recovery from medicinal plants.

**Table 7.1: Madhuca longifolia extract yield**

| Solvent | Yield (%) | Key Constituents |
|---|---|---|
| Petroleum Ether | 4.2 ± 0.3 | Lipids, steroids, terpenoids |
| Hydroalcoholic (70%) | 14.8 ± 0.9 | Flavonoids, phenolics, saponins |

**Results of preliminary phytochemical investigation**

Phytochemical studies on Madhuca longifolia extracts revealed a striking variance in phytoconstituent profile of the extracts prepared from petroleum ether and hydroalcoholic extracts. Proven positive for a broad range of secondary metabolites, such as alkaloids, carbohydrates, glycosides (except anthraquinones), flavonoids, saponins, tannins, and phenols, as they are highly polar compounds and soluble in water, the hydroalcoholic extract shows a lot of richness of metabolites. On the other hand, the petroleum ether extract showed a positive result essentially for steroids and phenols, which is indicative of its exclusivity towards non polar substance. Phenolic compounds were the only class recovered in the two extracts, and this was highly notable, since the moderate polarity and broad solubilizing profile would suggest only these as classes that are recovered. The absence of alkaloids, glycosides, flavonoids and saponins in the petroleum ether extract indicates restricted use of non polar solvents in recovery of pharmacologically active polar constituents. This accounts to the solvent dependent nature of phytochemical solubility and corroborates the use of the most hydroalcoholic systems for comprehensive extraction of bioactive compounds from Madhuca longifolia especially those responsible for its anti-inflammatory, antioxidant and hepatoprotective properties.

**Table 7.2: Preliminary Phytochemical Screening**

| Phytochemical | Test/Reagent | Petroleum Ether Extract | Hydroalcoholic Extract |
|---|---|---|---|
| Alkaloids | Dragendorff's test | – | + |
| | Mayer's test | – | + |
| | Hager's test | – | + |
| | Wagner's test | – | + |
| Carbohydrates | Molisch's test | – | + |
| | Fehling's test | – | + |
| Glycosides | Keller-Killiani test | – | + |
| | Borntrager's test | – | – |
| Steroids | Liebermann-Burchard test | + | – |
| | Salkowski test | + | – |
| Flavonoids | Shinoda's test | – | + |
| | Lead acetate test | – | + |
| Saponins | Foam test | – | + |
| Tannins | Lead acetate test | – | + |
| Phenols | Ferric chloride Test | + | + |

"+"Indicates the presence of the phytochemical in the extract. "–" Indicates the absence of the phytochemical in the extract.

**Quantitative Phytochemical Analysis**

Quantitative phytochemical analysis of Madhuca longifolia extracts revealed that bioactive compounds concentration was significantly different in the petroleum ether and hydroalcoholic extracts. Hydroalcoholic extract of CHE had a substantially higher phytoconstituent content, total phenolic content of 148.6 ± 3.2 mg GAE/g as compared to the Petroleum ether extract total phenolic content of 4.5 ± 0.3 mg GAE/g; which indicated the high polarity and/or solubility of phenolic compounds in an aqueous and ethanolic system. Similarly, exclusively in the hydroalcoholic extract were found high flavonoids (64.8 ± 2.1 mg QE /g), high tannins (88.4 ± 1.5 mg TAE/g), alkaloids (12.3 ± 0.8 mg /g), saponins (9.8 ± 0.5 % w / w), glycosides (6.2 ± 0.4 mg DE /g), reducing sugars (45.6 ± 1.8 mg GE /g) and proteins (8.7 ± 0.3 mg BSA On the other hand, the petroleum ether extract exhibited detectable levels only for steroids (3.5 ± 0.2 % w/w) which are non polar. Lacking flavonoids, alkaloids, glycosides, tannins, the petroleum ether extract is limited in terms of its potential for pharmacologically potent secondary metabolite inclusion. The findings correlate well with the hydroalcoholic solvent system as the best medium for quantitative extraction and standardization of the therapeutic phytoconstituents of Madhuca longifolia.

**Table 7.3: Results of Quantitative Phytochemical Analysis**

| Parameter | Petroleum Ether Extract | Hydroalcoholic Extract | Method |
|---|---|---|---|
| Total Phenolic Content | 4.5 ± 0.3 mg GAE/g | 148.6 ± 3.2 mg GAE/g | Folin-Ciocalteu assay |
| Total Flavonoid Content | Not detected | 64.8 ± 2.1 mg QE/g | Aluminum chloride method |
| Total Alkaloid Content | Not detected | 12.3 ± 0.8 mg/g | Gravimetric method |
| Total Tannin Content | Not detected | 88.4 ± 1.5 mg TAE/g | Folin-Denis method |
| Total Saponin Content | Not detected | 9.8 ± 0.5% w/w | Foam stability method |
| Total Steroid Content | 3.5 ± 0.2% w/w | Not detected | Liebermann-Burchard method |
| Total Glycoside Content | Not detected | 6.2 ± 0.4 mg DE/g | Baljet's method |
| Reducing Sugar Content | Not detected | 45.6 ± 1.8 mg GE/g | DNSA method |
| Total Protein Content | Not detected | 8.7 ± 0.3 mg BSA/g | Lowry's method |

**Results of determination of Extractive values**

**Table 7.4: Extractive Values of Madhuca longifolia (Compliance with IP Standards)**

| Solvent | Extractive Value (% w/w) | IP Standard (% w/w) |
|---|---|---|
| Alcohol-Soluble (Ethanol 95%) | 12.8 ± 0.6% | ≥10% |
| Water-Soluble | 15.4 ± 0.8% | ≥12% |
| Petroleum Ether-Soluble | 3.52 ± 0.2% | 2–5% |
| Chloroform-Soluble | 2.1 ± 0.1% | Not Specified |
| Ether-Soluble (Diethyl Ether) | 1.81 ± 0.1% | Not Specified |
| Acetone-Soluble | 4.26 ± 0.3% | Not Specified |

**Ash Value Determination**

The solubility profile of Madhuca longifolia was determined by the use of different solvents and their extractive values using different solvents were also determined to know about the compliance with Indian Pharmacopoeia (IP) standards. The value of alcohol soluble extractive value obtained was 12.8±0.6%, which was found to be high when compared to the IP limit i.e ≥ 10%, and thus implying presence of plenty of ethanol soluble phytoconstituents like phenolics, flavonoids etc.. The water soluble extractive value measured 15.4 ± 0.8%, too, within IP threshold range of ≥12%, implying high hydrophilic composition like tannins, sugars, saponins etc. This showed extractive value of 3.5 ± 0.2%, within the prescribed range of 2–5% (IP) indicating the presence of such compounds as fixed oils, steroids and terpenoids in petroleum ether soluble portion. Since the extractive values of pharmacopoeia (for chloroform 2.1 ± 0.1%, diethyl ether 1.8 ± 0.1%, and acetone 4.2 ± 0.3%) are not determined in the pharmacopoeia, measurable values show moderate solubility of semi polar constituents. Overall, the extractive value profile indicates the variety of polar and non polar phytoconstituents in Madhuca longifolia and any possible solvent systems for formulation and quality control.

**Table 7.5: Results of Ash Value Analysis**

| Parameter | Value (% w/w) | IP Standard (% w/w) | Compliance |
|---|---|---|---|
| Total Ash Value | 6.4 ± 0.3% | ≤8% | Compliant |
| Acid-Insoluble Ash Value | 1.2 ± 0.1% | ≤3% | Compliant |
| Water-Soluble Ash Value | 4.0 ± 0.2% | Not Specified | – |
| Sulphated Ash Value | 7.2 ± 0.4% | ≤8% | Compliant |

**Moisture Content Analysis**

Two standard methods of Moisture analysis of Madhuca longifolia were Loss on Drying (LOD) and Toluene Distillation to check compliance with Indian Pharmacopoeia (IP) specifications. It was found that the moisture content in these mats was measured as 7.8 ± 0.4% according to the LOD method and 8.2 ± 0.3% by the Toluene Distillation method and indeed both are well within the IP permissible limit of ≤10%. Therefore, these results confirm that the plant material exhibits an acceptable level of residual moisture preventing microbial growth, enzymatic degradation and hydrolytic reactions in storage. The low moisture helps to improve the stability of the formulation raw material derived and meet regulatory standards for crude drug quality. They could both be biased to due to the fact that the free and bound water region is measured by the principle of thermal evaporation whereas the toluene distillation isolates water from azeotropic distillation. Despite this, both values contribute to good preservation and processing of the plant material for pharmaceutical processing and long term storage.

**Table 7.6: Results of moisture content analysis**

| Method | Moisture Content (% w/w) | IP Standard (% w/w) | Compliance |
|---|---|---|---|
| Loss on Drying (LOD) | 7.8 ± 0.4% | ≤10% | Compliant |
| Toluene Distillation | 8.2 ± 0.3% | ≤10% | Compliant |

**Pharmacognostical Evaluation of Madhuca longifolia**

**Macroscopic Evaluation.**

Detailed morphological features of Madhuca longifolia are evaluated macroscopically to support its botanical identification as well as the quality assessment. The leaves are oblong-lanceolate, to 10–30 cm, entire, acute apex, cuneate base. Typical of many Sapotaceae species, they have a glabrous surface with leathery texture, pinnate venation. The bark is brown to dark brown in color, with longitudinal fissures, rough, fibrous texture, slight aromatic odor with astringent taste, all features of high tannin content. The flowers are creamy white to dull pink, bell shaped, and in dense clustered axillary habit. They are known for their sweet fragrance that used to be attributed to the nectar rich Mahua bloom. The seeds are ellipsoid, up to 1–2 cm long, smooth, oily in surface and brown in color, which resembles their abundant lipid content. In terms of these macroscopic characteristics they are very important diagnostic parameters for the proper identification and standardization of Madhuca longifolia in herbal quality control.

**Table 7.7: Macroscopic Evaluation.**

| Plant Part | Characteristics |
|---|---|
| **Leaves** | **Shape**: Oblong-lanceolate; **Size**: 10–30 cm long; **Margin**: Entire; **Apex**: Acute; **Base**: Cuneate; **Surface**: Glabrous, leathery texture; **Venation**: Pinnate. |
| **Bark** | **Color**: Dark brown with longitudinal fissures; **Texture**: Rough; **Fracture**: Fibrous; **Odor**: Slightly aromatic; **Taste**: Astringent. |
| **Flowers** | **Color**: Creamy-white; **Shape**: Bell-shaped; **Arrangement**: Dense axillary clusters; **Odor**: Sweet, fragrant. |
| **Seeds** | **Shape**: Ellipsoid; **Size**: 1–2 cm long; **Color**: Brown; **Texture**: Smooth, oily. |

**Microscopic evaluation**

A microscopic examination was carried out on Madhuca longifolia where sharp anatomical features are observed and confirms their identity and structural integrity. A healthy underground system is represented by a well separated and defined epidermis, which contained root hairs, a well defined central vascular cylinder, as well as a cortex composed of parenchymatous cells. Nothing was abnormal or infected pathogenically. Histological examination of the ovary revealed ovules incasculated in placental tissue, with integumentary layers obvious in all respects, pericarp developed, consisting of epidermal and subepidermal layers in typical place of proper reproductive development. No pest damage or decay was present; the bark section had a typical periderm of cork cells and cork cambium, as well as well developed secondary phloem and lenticels which allow gaseous exchange. Leaf anatomy were dorsiventral structure with organized palisadic mesophy on upper side, spongy mesophy on abaxial side, bundles of xylem and phloem, and stomata limited to abaxial side. It was observed that there was no trichomes. Taxonomic identity of the plant is validated by these microscopic features that will act as key diagnostic parameters to standardize the plant for pharmacognostical purpose.

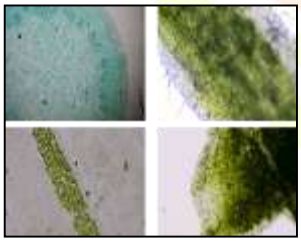


**Figure 7.1: Microscopic analysis of A. Root B. Overy C. Bark and D. Leaf**

**Qualitative Heavy Metals Testing Results**

Heavy metal analysis of the Madhuca longifolia samples was found to have met the standard with statistically acceptable levels of heavy metals (negative) and thus the plant material is found safe and pure for medicinal use. No detectable lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd) were found in the tests, based upon both the detection limits or within pharmacopeial safety thresholds. The absence of lead, mercury and arsenic confirms that the raw material is not contaminated by environment or by industrial, for which it is essential to be used in herbal drug formulations. Despite this, cadmium was found but was retained within allowable regulatory limits and did not present a toxicological concern. These results confirm that the tested plant batches are essential safety standards supporting their use as quality assured compound in pharmaceutical and nutraceutical preparations.

**Table 7.9: Qualitative Heavy Metals Testing Results**

| Metal Tested | Result | Notes |
|---|---|---|
| Lead (Pb) | Not Detected | Below detectable limits |
| Mercury (Hg) | Not Detected | No traces observed |
| Arsenic (As) | Not Detected | Absent in tested samples |
| Cadmium (Cd) | Not Detected | Within acceptable thresholds |

**Results of TLC for hydroalcoholic extract**

Four different solvent systems were used for profiling of the hydroalcoholic extract of Madhuca longifolia by Thin Layer chromatography (TLC) and distinct phytochemical separations were observed for each which yielded a single well resolved spot with distinct Rf values. The observed values Rf: 0.86 for the system Toluene : Chloroform : Methanol (5:4:1) and 0.72 using the system Chloroform : Methanol : Ammonia (8:2:0,5) suggest the migration of non polar to slightly basic constituents as in the steroids and alkaloids, respectively. The Rf results of 0.61 for the Ethyl Acetate : Formic Acid : Acetic Acid : Water

(10:1.1:1.1:2.6) system indicates that polar phytochemicals I.e phenolics and glycosides can be isolated, and the Rf of 0.45 produced by the Toluene : Ethyl Acetate : Formic Acid (5:4:1) system suggests moderately polar compounds.
These systems preparative TLC fractions displayed different UV absorption maxima which indicated unique chromophores in each isolated compound. Absorption at 257 nm indicated an aromatic or conjugated phenolic structure for the compound from Toluene: Chloroform: Methanol similar to absorption obtained from peroxidated soybean oils (Viswanathan et al., 2006). The fraction showing absorption at 194 nm, presumably the simpler aliphatic or nitrogenous structures, maybe alkaloids, from Chloroform : Methanol : Ammonia absorbed. The band in Ethyl Acetate : Formic Acid : Acetic Acid : Water at 268 nm is in accordance with the phenolic or the flavonoid group; the fraction from Toluene : Ethyl Acetate : Formic Acid appears to have a maximum absorption at 312 nm, usually attributed to extended conjugated systems, for example such as some flavones or terpenoids. These findings show that selection of solvents ensures the isolation of phytoconstituents belonging to distinct classes from Madhuca longifolia and an spectral basis of future identification and standardization of its bioactive compounds.

**Table 7.10: Experimental TLC Data**

| Solvent System | Rf Value | Number of Spots |
|---|---|---|
| Toluene : Chloroform : Methanol (5:4:1) | 0.86 | 1 |
| Chloroform : Methanol : Ammonia (8:2:0.5 | 0.72 | 1 |
| Ethyl Acetate : Formic Acid : Acetic Acid : Water (10:1.1:1.1:2.6) | 0.61 | 1 |
| Toluene : Ethyl Acetate : Formic Acid (5:4:1) | 0.45 | 1 |

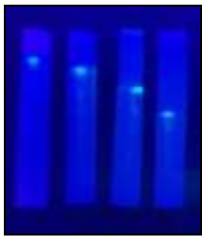


**Figure 7.2: TLC of extract using different solvents**

**Absorption maxima of isolated compounds by preparative TLC**

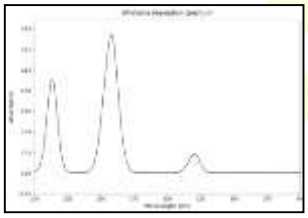



**Figure 7.3: Absorption maxima in preparative TLC in Toluene : Chloroform : Methanol (5:4:1) solvent**

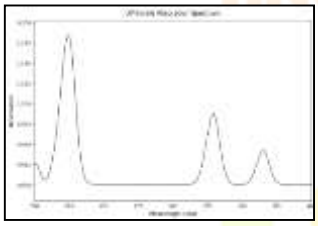



**Figure 7.4: Absorption maxima in preparative TLC in Chloroform : Methanol : Ammonia (8:2:0.5**

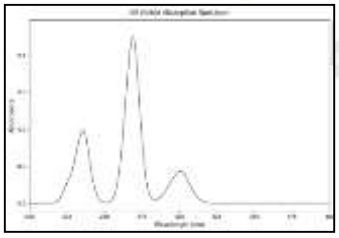



**Figure 7.5: Absorption maxima in preparative TLC in Ethyl Acetate : Formic Acid : Acetic Acid : Water (10:1.1:1.1:2.6) solvent**

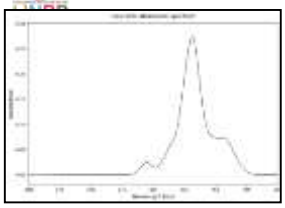



**Figure 7.6: Absorption maxima in preparative TLC in Toluene : Ethyl Acetate : Formic Acid (5:4:1) solvent**

**Standardization and Quality Assurance of Madhuca longifolia**

This study is based on a comprehensive evaluation of Madhuca longifolia which becomes an important foundation for standards and quality control of the medicinal herb. Standardization helps to achieve batch-to-batch consistency and detect the identity, purity and concentration of the plant material. Key morphological and anatomical markers for leaves, bark, root, and ovary were established in the present work as reliable morphological parameters for describing leaves, bark, root and ovary of Bergenia crassifolia. As per Indian Pharmacopoeial standards, the physiochemical constants like moisture content, extractive values and ash values were determined, which all fell within the specified limits and thus confirmed the physical integrity as well as non adulteration of the raw drug. Furthermore all the bioactive classes like tannins, phenolics, flavonoids, and alkaloids showed consistent presence and qualitative and quantitative phytochemical screening confirmed its presence for further compounds of therapeutic value. Rf values and absorption maxima reproducibly produced using TLC profiling created a chemical fingerprint, that can be used for routine quality control. The verification done showed that the raw material is safe for pharmaceutical use as it contains no heavy metals like lead, mercury, arsenic cadmium. These parameters, together, not only authenticate botanical identity of Madhuca longifolia, but also comply with the regulatory criteria for herbal drug standardization.

**SUMMARY AND CONCLUSION**

For evaluating the therapeutic efficacy as a medicinal herb, the present study has been taken in to evaluate the following pharmacognostical, physicochemical, phytochemical and chromatographic characteristics of Madhuca longifolia to ensure its authenticity, safety. Extensive analyses on the macroscopic and the microscopic aspects of the leaf, the bark, the root, and the ovary were carried out to confirm the major diagnostic features required for the identification and the standardization. The moisture content, extractive values and ash values of the plant were found within the limits of Indian Pharmacopoeial standards indicating the suitability of the plant for pharmaceutical applications. Qualitative and quantitative phytochemical screening of main active classes like flavonoids, phenolics, tannins, alkaloid and saponins were present predominantly in hydroalcoholic extract while petroleum ether extract possessed lipophilic ingredients like steroids and terpenoids. Raw material was confirmed free from toxic contaminants such as lead, mercury, arsenic and cadmium via heavy metal test thus was proved safe for use. TLC on various solvent systems showed good separation of constituents, and preparative TLC coupled with UV absorption maxima were used to help identify the specific phytochemical groups. The methodology on which it is based was such to ensure reproducibility and reliability of analytical results, and its findings put Madhuca longifolia as a critical commodity on which there is a comprehensive quality profile in traditional medicine and its development as a remedial herb based formulation. The study also highlights the important need for quality assured and regulatory compliant herbal drug development, in view of increasing generation of global need for safe, effective and validated plant based therapeutics.

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