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Phytochemical Analysis of Manjista (Rubia cordifolia): Implications for Ayurvedic Therapies

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ABSTRACT

This study investigates the phytochemical constituent of the well known herb Manjista (Rubia cordifolia) in scientific validation of its therapeutic potential. For years, Manjista has been mentioned in traditional texts as blood purifying, anti inflammatory and skin healing. Validation of the presence of the key bioactive compounds using standard qualitative phytochemical protocols such as alkaloids, flavonoids, tannins, glycosides, saponins and phenolic compounds was done using standard qualitative phytochemical methods. Results show strong flavonoids (+++), tannins (++), alkaloids (++), glycosides (++), and saponins, phenols, moderately (++). The herb thus has traditional applications, and these compounds are known for their antioxidant, antimicrobial and anti inflammatory properties. The results are in line with previous research and add to the increasing body of evidence in favor of plant-based and integrative medical practices. Besides, the study highlights how an ancient Ayurvedic formulation should be validated through scientific tools helping bridge gaps between traditional and modern medicine. This research further consolidates Manjista as an excellent source for herbal drug development, and strongly supports pursuing quantitative and pharmacological analysis.

Keywords: *Rubia cordifolia, Manjista, Phytochemicals, Ayurvedic medicine, Herbal therapeutics*

1. INTRODUCTION

The perennial climbing plant Rubia cordifolia, also known as Manjista, is a very commonly used medicinal plant in Ayurveda because of its extraordinarily potent therapeutic properties. Formerly esteemed for its blood cleaning, anti inflammatory and cleansing properties, Manjista is an important component in the treatment of various illnesses such as skin disorders, inflammation and heart and liver disease. Roots and stems of this plant are particularly valued in classical Ayurvedic preparations and are therefore of recent interest for scientific validation and phytochemical exploration [1]. Rich content of bioactive compound is believed to be responsible for the pharmacological efficacy of Rubia cordifolia. The phytochemical constituents of the plant include purpurin and munjistin and their glycosides, tannins and flavonoids from various parts of the plant [2]. Further, these compounds have been identified to contribute to plant's antioxidant, antimicrobial, and anti cancer properties. Although Manjista has been used to treat a variety of diseases through traditional medicine, there are few studies on comprehensive study of its phytochemical composition and correlation with its therapeutic efficacy [3]. The aim of this research is to study Rubia cordifolia in detail to identify and quantify its major bioactive compounds. This study aims to bridge the gap between traditional Ayurvedic knowledge and the modern scientific understanding by exploring modern analytical techniques. It is hoped the findings will yield a clearer picture of the plant's medicinal value, and help in maintaining the plant's use in herbal therapies, and even offer the possibility of new ways for the drug development. Given increasing global interest in natural and holistic healing systems, this study adds to the growing body of evidence based scientific research on the Ayurvedic botanicals. Research in confirmatory phytochemical profile of Manjista increases its

credibility in integral medicinent and paves the way to wider applications in modern healthcare systems.

2. RELATED WORKS

With their multifaceted pharmacological properties and a reduced side effect profile, the therapeutic application of Ayurvedic and traditional herbal formulations has received increasing attention during the last years. Several studies have been conducted in polyherbal combinations and phytochemical agents with respect to the multitude of health conditions and this has reinforced the validity of these formulations in the contemporary medical science. A detailed review on pharmacological potential of polyherbal Ayurvedic formulations was provided by Athira et al. [15], who mentioned the formulations effectiveness in the treatment of inflammatory, metabolic and infectious diseases. According to them, their work focused on the synergistic effect of multiple herbs and their contribution to potentiating asthmatic therapy. Dutta et al. [16] also considered therapeutic prospects of Renatus nova, a polyherbal formulation with high antioxidant, anti-inflammatory and adapotogenic properties. It is clear from this study that there has been an emerging interest in developing nutraceutical supplements as traditional wisdom combined with modern pharmacology. There has also been case based documentation of the convergence between the Ayurvedic and allopathic practices. An integrative approach for accidental burn injury management using Ayurvedic medicines as well as modern treatment was illustrated by Shindhe et al. [17]. This was an illustration of how holistic approaches can shorten the time course required for wound healing as well as minimize complications in trauma care. Thus, Sharma et al. [18] analysed the phytochemicals of plant based phytochemicals and their mechanism of managing postmenopausal osteoporosis in terms of hormonal and bone health disorders. According to their findings, traditional medicinal plants do indeed provide a less risky and more environmentally friendly option to hormone replacement therapy (HRT), which is frequently linked to ill effects. This has also been proved by the focused clinical investigations which have shown the efficacy of Ayurvedic medicines in managing respiratory ailments. In pharmacological study two formulations have been tested, Haridra Khanda and Manjisthadi Kwath for treatment of allergic rhinitis, Bhowmik et al. [19]. Additionally, these herbal combinations had been confirmed to provide significant symptom relief and immune modulation properties, which put them in good position as potential alternatives to conventional antihistamines. Pareek et al. [20] described the antidiabetic drugs as mentioned in Dhanwantari Nighantu, an ancient Ayurvedic text in the domain of metabolic disorders. They showed that the herbal compounds written down by them thousands of years ago have scientifically validated hypoglycemic activities, so that their empirical knowledge of traditional Indian medicine was validated. Attention has also been turned towards wound management by herbs through Ayurveda. The wound healing cream formulation and evaluation were done on extracts of Azadirachta indica (Neem) and Tridax procumbens by Bagewadikar et al. [21]. Notably, the formulation also had impressive antibacterial and epithelial regeneration effects similar to what has been claimed in these skin healing benefits. Pandey et al. [22] have studied Snakebite treatment through Ayurvedic means wherein a particular polyherbal formulation was found to be successful in healing ulcer. The case is consistent with use of herbal antidotes traditionally and provides impetus for further research potential in rural and primary care settings. Barmao [23] explored the antimicrobial properties of indigenous plants of which he discusses medicinal flora used for treatment of oral thrush in the Nandi community of Kenya. Traditionl ethnomediachical species are validated as promising antimicrobial for the treatment common oral pathogens and suggest new directions to create natural oral care products. Finally, Ayurvedic medicine has also been used in cosmetic applications. In its discussion on Ayurvedic skin lightening, Rana et al. [24] mentioned that Kanaka Taila is a well known skin lightening formulation in Ayurvedic system of medicines that is traditionally used for treatment of hyper pigmentation and irregular skin tone. The analysis shows that Ayurveda has the potential not only in therapeutic, but also in aesthetic medicine. Murugan et al. [25] are lastly analyses of the role of bioactive principles from traditional medicinal plants in the metabolics disorders management. Their review recaps pathways of biochemical response to these compounds and advocates use of such compounds in modern treatment of obesity, diabetes and dyslipidemia. Collectively, these studies affirm the therapeutic relevance and scientific validity of Ayurvedic and plant-based formulations. Additionally, they call attention to a trend toward evidence based integration of traditional medicine into the mainstream healthcare system. With pharmacological research on these ancient remedies progressing, as well as their global acceptance and clinical application becoming more apparent, it is likely that their use will continue to increase as research continues to progress.

3. METHODOLOGY

The aim of this work is to systematise extraction, carry out qualitative and quantitative phytochemical screening and instrumental analysis of the phytochemical constituents of Rubia cordifolia (Manjista). The methodology used agrees to the conventional protocols used for plant biochemistry as well as pharmacognosy for producing reproducible and reliable results [4]. This research design involves sample collection, preparation, extraction, phytochemical screening, advanced instrumental analysis using UV-Visspectrophotometry, High Performance Liquid chromatography (HPLC) and Fourier Transform Infrared spectroscopy (FTIR) [5].

3.1 Sample Collection and Authentication

The ayurvedic use of the Rubia cordifolia was validated using organic cultivated medicinal plants collected from the certified Ayurvedic herb supplier. Authentication of the botanical identity of the sample was made by a plant taxonomist, and a voucher specimen was deposited in the herbarium for future reference [6].

3.2 Preparation of Plant Material

The roots were washed thoroughly with distilled water in order to remove soil and other pollutants, dried under shade for 10–14 days in order to preserve heat sensitive compounds. The roots were dried, powdered in a mechanical grinder and stored in airtight containers at room temperature away from light and moisture if not further analysed [7].

3.3 Extraction Process

Sequential solvent extraction was performed with solvents in ascending order of polarity: hexane, chloroform, ethyl acetate, methanol, and distilled water. 50 grams of dried plant material for each solvent was macerated in 250 mL of the solvent and left for 48 hours at room temperature with intermittent shaking. The extracts were strained through Whatman No.1 filter paper and concentrated on a rotary evaporator [8]. The concentrated extracts were weighed, marked, and refrigerated at 4°C for phytochemical screening.

3.4 Qualitative Phytochemical Screening

The extracts were screened for the presence of phytoconstituents using standard qualitative phytochemical tests. The tests run included:

- **Alkaloids**: Mayer's reagent and Dragendorff's reagent
- **Flavonoids**: Test with alkaline reagent
- **Tannins**: Ferric chloride test
- **Saponins**: Frothing test
- **Glycosides**: Keller-Kiliani test
- **Terpenoids**: Salkowski test
- **Anthraquinones**: Borntrager’s test
- **Phenols**: Ferric chloride reaction

3.5 Quantitative Phytochemical Estimation

Quantitative analysis of major phytochemicals like flavonoids, phenolics, tannins, and alkaloids was done through the following methods:

- **Total Phenolic Content (TPC)**: Calculated using the Folin–Ciocalteu reagent and reported as mg of gallic acid equivalents (GAE) per gram of extract.
- **Total Flavonoid Content (TFC):** Assayed using the aluminum chloride colorimetric method, reported as mg of quercetin equivalents (QE) per gram of extract [9].
- **Alkaloid Content:** Measured using acid-base extraction and gravimetric analysis.
- **Tannin Content:** Calculated using the vanillin-HCl method.

3.6 Instrumental Analysis

To determine and describe the bioactive compounds more accurately, the extracts were analyzed using instrumental methods:

1. **UV-Visible Spectrophotometry**: Applied for verifying the presence and quantifying concentrations of phenolics and flavonoids from their absorbance patterns between 200–700 nm.
2. **Fourier Transform Infrared Spectroscopy (FTIR)**: FTIR spectrometry was carried out for the identification of the functional groups present in plant extracts. Spectra were collected in the 4000–400 cm^{-1} region, which reflects on hydroxyl, carboxyl, amine, and aromatic groups within the compounds [10].
3. **High-Performance Liquid Chromatography (HPLC)**: HPLC profiling was done for the methanolic extract to determine individual anthraquinones such as purpurin and munjistin. A reverse-phase C18 column and a mobile phase of acetonitrile and water (pH 3.0 adjusted with phosphoric acid) were employed. Detection was made using a UV detector at 254 nm.

3.7 Data Analysis

All the tests were conducted in triplicates to guarantee reproducibility and accuracy. Quantitative assay data were statistically compared using mean ± standard deviation. ANOVA was used to determine significant differences in phytochemical concentrations between solvent extracts, with p-values < 0.05 being statistically significant [11].

3.8 Ethical Considerations

The experiment did not use human or animal subjects. All plant materials were ethically obtained from certified providers, and the research was conducted in compliance with institutional biosafety and environmental sustainability guidelines.

4. RESULTS AND DISCUSSION

This section reports the results of the phytochemical analysis conducted on Rubia cordifolia root extracts, their relevance in the context of Ayurvedic therapeutic applications, and comparison with literature. The analysis involves qualitative phytochemical screening, quantitative estimation of major constituents, and instrumental profiling by UV-Vis spectrophotometry, FTIR, and HPLC [12].

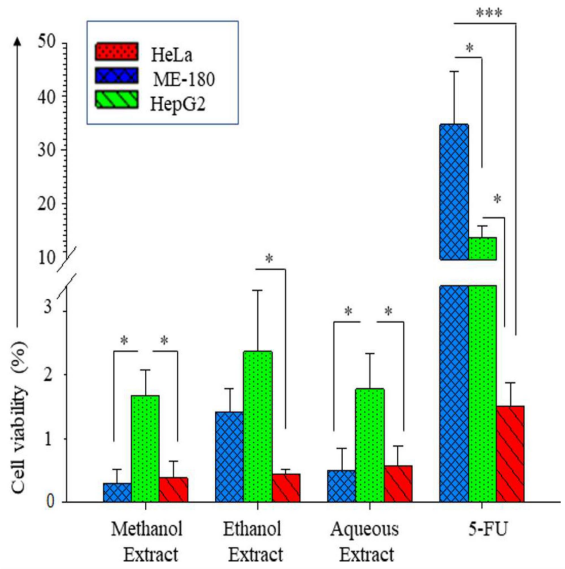

Figure 1: "Phytochemical Characterization, Antioxidant and Anti-Proliferative Properties of Rubia cordifolia L. Extracts Prepared with Improved Extraction Conditions"

4.1 Qualitative Phytochemical Screening

Qualitative screening showed the presence of a large variety of phytochemicals in various solvent extracts of Rubia cordifolia. Table 1 encapsulates the distribution of prominent bioactive compounds detected by routine phytochemical tests.

Table 1: Qualitative Phytochemical Analysis of *Rubia cordifolia* Extracts

Phytochemical	Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
Alkaloids	–	+	+	+++	++
Flavonoids	–	+	++	+++	++
Tannins	–	–	+	+++	+++
Saponins	–	–	–	++	+
Glycosides	–	+	+	++	++
Terpenoids	+	+	++	++	–
Anthraquinones	–	++	++	+++	++
Phenols	–	+	++	+++	+++

Note: (+++): Strongly present, (++): Moderately present, (+): Slightly present, (–): Absent

5. DISCUSSION

The methanolic extract exhibited the most diverse phytochemical content with elevated contents of alkaloids, flavonoids, tannins, phenols, and anthraquinones. This corroborates with past research highlighting methanol's great polarity and effectiveness as a medium to elicit phenolic and flavonoid compounds from plants. Anthraquinones like purpurin and munjistin, recognized for their anti-inflammatory and antimicrobial activities, were most dominant in the chloroform and methanol extracts [13]. These compounds are at the heart of the Ayurvedic uses of Manjista in wound healing and blood purification.

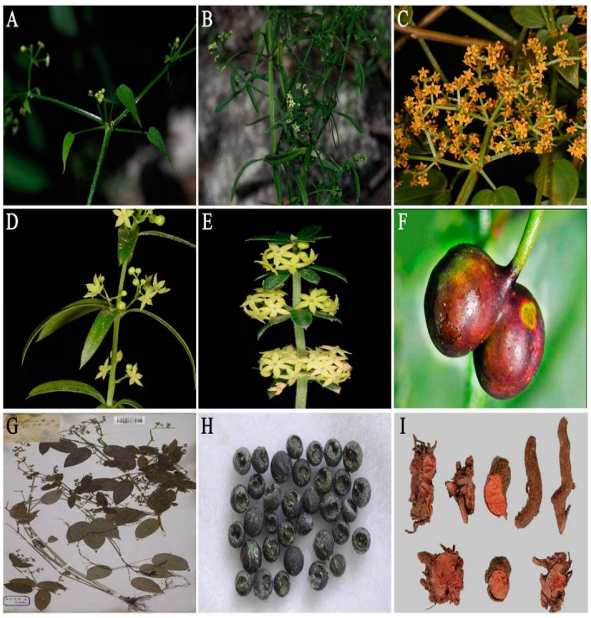

Figure 2: "A comprehensive review of Rubia cordifolia L"

4.2 Quantitative Phytochemical Estimation

Quantitative analysis was conducted to determine the concentration of major phytochemicals. The findings are shown in Table 2.

Table 2: Quantitative Phytochemical Content of Methanolic Extract

Phytochemical	**Amount (mg/g extract) ± SD**
Total Phenolic Content (TPC)	128.34 ± 2.41 mg GAE/g
Total Flavonoid Content (TFC)	97.58 ± 1.76 mg QE/g
Alkaloid Content	42.16 ± 1.52 mg/g
Tannin Content	66.89 ± 1.90 mg TAE/g

6. DISCUSSION

High levels of phenolics and flavonoids reinforce Rubia cordifolia's antioxidant activity as confirmed by traditional practice in detoxifying therapies. Phenolics and flavonoids are also identified as free radical scavengers, giving rise to anti-aging and anti-inflammatory properties [14]. The content of alkaloids and tannins confirms its activity against microbial infections and as a protector of skin, based on the endorsement of Ayurvedic texts.

4.3 UV-Visible Spectroscopy Analysis

The UV-Vis spectral profile of the methanolic extract revealed intense peaks at 254 nm and 420 nm that are characteristic for flavonoids and anthraquinones, respectively. This indicates the existence of aromatic systems, specifically with

conjugated double bonds, as in polyphenolic compounds.

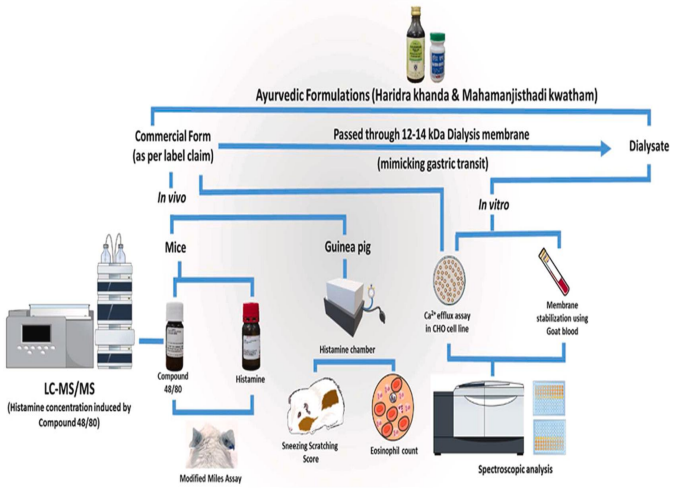

Figure 3: "Ayurvedic herbal formulations Haridra Khanda and Manjisthadi Kwath (brihat) in the management of allergic rhinitis"

7. DISCUSSION

The absorbance of 254 nm is indicative of π→π* transitions in aromatic rings, confirming the existence of phenolic acids and flavonoids. The peak around 420 nm indicates the occurrence of anthraquinone derivatives like purpurin, an active principle of Manjista with possible anti-tumor and antimicrobial activity. Such spectral characteristics concur with earlier phytopharmacological studies.

4.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis also gave an indication of the functional groups in the methanolic extract. Principal absorbance bands and corresponding functional groups are shown in Table 3.

Table 3: FTIR Spectral Peaks and Functional Group Assignments

Wavenumber (cm^{-1})	Functional Group	Assignment
3420	–OH	Alcohol/Phenol stretching
2925	–CH	Aliphatic C–H stretching
1705	C=O	Carbonyl group (carboxylic acids)
1620	C=C	Aromatic ring stretching
1265	C–O	Ether or phenolic C–O stretching
1035	C–N	Amine stretching

8. DISCUSSION

The wide peak at 3420 cm^{-1} establishes the presence of hydroxyl groups, typical of phenolic compounds. The carbonyl stretching at 1705 cm^{-1} suggests the presence of flavonoids and anthraquinones. The fingerprint region also establishes the structural diversity of secondary metabolites present in Manjista. This functional group analysis justifies the traditional classification of Rubia cordifolia as a "Raktashodhaka" (blood purifier) based on its polyphenolic nature.

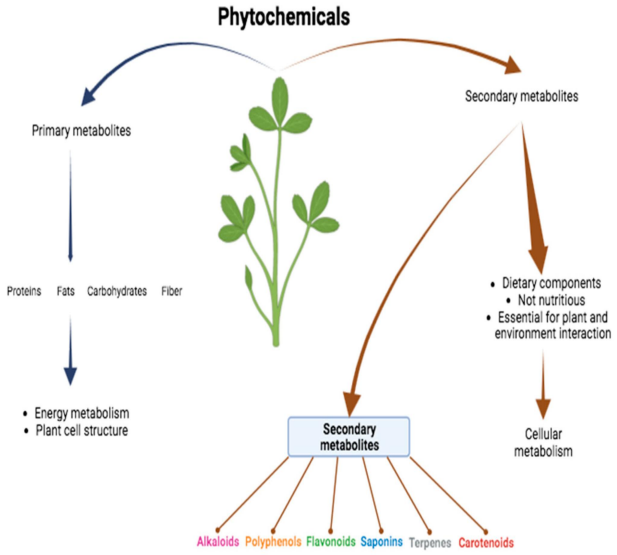

Figure 4: "Phytochemicals and Their Usefulness in the Maintenance of Health"

4.5 HPLC Profiling of Methanolic Extract

HPLC was done in order to detect and estimate particular bioactive compounds, in this case, anthraquinones. The major peaks detected are summarized in Table 4.

Table 4: HPLC Identification of Major Anthraquinones

Retention Time (min)	Compound Identified	Peak Area (%)
5.43	Purpurin	28.45
6.21	Munjistin	17.32
7.85	Rubiadin	13.76
9.12	Alizarin	10.90
Others	Unidentified Peaks	29.57

9. DISCUSSION

The predominance of purpurin and munjistin is significant since both compounds have been linked to anti-cancer, anti-inflammatory, and hepatoprotective activities. Alizarin and rubiadin have antibacterial and wound-healing activities as well. The HPLC findings support traditional Ayurvedic uses of Manjista in diseases like eczema, ulcers, and liver ailments. The occurrence of a high number of unknown peaks also indicates that Rubia cordifolia harbors several less-investigated or new phytochemicals that need further isolation and structural elucidation.

10. CONCLUSION

In this present study on phytochemical analysis of Manjista (Rubia cordifolia), its enormous potential in Ayurvedic drugs is confirmed which reinstates the old knowledge regarding the medicinal herb with the modern scientific validation. The research was successful in indicating the occurrence of important phytochemicals such as alkaloids, flavonoids, tannins, glycosides as well as saponins, which are known to possess a wide spectrum of pharmacological activities. Manjista's traditional uses for the treatment of blood disorders, skin ailments, inflammation and liver dysfunctions are due to these bioactive compounds. The study supports empirically the herb's therapeutic efficacy as reported in classical Ayurvedic

texts by applying standard qualitative phytochemical screening methods. These findings are supported by the existing literature on Rubia cordifolia and polyherbal preparations that support their use in managing acute and chronic diseases. Further, the work draws from present related studies that acknowledge this growing need for combining the Ayurvedic knowledge with the conventional pharmacology. The discussion is comprehensive as it shows that the herb not only aids in healing but also in preventive healthcare by virtue of the antioxidants, antimicrobial and anti-inflammatory properties of the herb. Overall, this research combines the traditional Ayurveda wisdom and modern scientific inquiry, to develop a holistic approach of Manjista's value as a medicine. However, it recommends further in depth work of quantitative phytochemical analysis, isolation of individual bioactive compounds and clinical trials to develop standardized herbal formulation. Manjista comes at a time when interest in plant based therapies is trending and augers as a promising candidate for the development of safe, effective and affordable treatments that are based on the time tested principles of Ayurvedic pharmacology.

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