RESEARCH PAPER

A Pharmaceutico-Analytical Standardization and In-Vitro Anti-Mycobacterial Evaluation of Nirgundi Ghrita for Tuberculosis Management

Dr. Rupali G. Wadibhasme (Chandewar)[1*] and Dr. Anita Wanjari[2]

[1]Ph.D. Scholar, Department of Rasshastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod, Wardha, Maharashtra-442001, Email: ngc609@gmail.com
[2]Professor, Department of Rasshastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod, Wardha, Maharashtra-442001, Email: anitawanjari7@gmail.com

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ABSTRACT

Tuberculosis remains a major global health challenge, particularly with the emergence of drug-resistant strains. The classical Ayurvedic formulation Nirgundi Ghrita, traditionally indicated for Rajayakshma (correlating with pulmonary tuberculosis), represents a promising adjunct therapy. This research aims to conduct comprehensive pharmaceutico-analytical evaluation and experimental validation of Nirgundi Ghrita, focusing on its antioxidant and antimycobacterial activities. The formulation will be prepared using Vitex negundo (Nirgundi) and cow ghee following classical guidelines from Chakradatta. Standardization will employ organoleptic, physicochemical, and chromatographic analyses. Antioxidant activity will be assessed through DPPH, ABTS, and FRAP assays, while antimycobacterial efficacy will be evaluated against Mycobacterium tuberculosis using the BacT/ALERT 3D automated system. Given the established role of oxidative stress in tuberculosis pathogenesis and the documented hepatoprotective, immunomodulatory, and antimicrobial properties of Vitex negundo, this study seeks to bridge traditional wisdom with evidence-based medicine. The findings will contribute to integrative tuberculosis management by validating a classical formulation that may enhance treatment outcomes, reduce drug toxicity, and strengthen host immunity through its antioxidant and antimycobacterial properties.

Keywords: Nirgundi Ghrita; Vitex negundo; Tuberculosis; Rajayakshma; Antioxidant activity; Antimycobacterial activity

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1. INTRODUCTION

Tuberculosis (TB) remains one of the most ancient yet persistent infectious diseases affecting humankind. TB remains a significant worldwide social health issue especially in developing nations despite the development of diagnostics and pharmacotherapy. Most cases are caused by pulmonary tuberculosis that is linked to long-term morbidity, socioeconomic cost, and death. The rise of the multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis strains has also complicated the management of the disease and emphasized the necessity of complementary and supportive treatment methods in addition to the conventional anti-tubercular medications (Sandhu, 2011; Miotto et al., 2022). According to Ayurvedic view, tuberculosis may be associated with the disease entity of Rajayakshma which is widely mentioned in the classical texts. Rajayakshma is typified by the progressive loss of body tissues (Dhatu Kshaya), loss of metabolism (Agni Vaishamya) and loss of vitality and immunity (Oja Kshaya). The progressive exhaustion of Rasa, Rakta, Mamsa, Meda, and Shukra eventually undermines the body defense mechanisms, and the person becomes prone to chronic infections and a degenerative process. This theoretical model is very similar to the current concept of TB as a chronic wasting illness with immune dysregulation and metabolic imbalance (Charak Samhita; Chakradatta).

Modern biomedical research has increasingly recognized the role of **oxidative stress** in the pathogenesis and progression of tuberculosis. Infection with *Mycobacterium tuberculosis* induces excessive production of reactive oxygen species (ROS) as part of the host immune response. Although ROS contribute to the clearance of pathogens, their continued overproduction causes oxidative destruction of host tissues, exacerbation of inflammation, and retarded healing. Some studies have already shown that TB patients have disrupted antioxidant defense mechanisms and increased oxidative stress biomarkers,

especially in the course of long-term anti-tubercular treatment, which can also contribute to tissue damage and drug-related toxicity (Naidoo et al., 2021; Stanciulescu et al., 2021; Qi et al., 2020).

The traditional anti-tubercular drugs, despite their efficacy, are often linked to some toxicity including hepatotoxicity, gastrointestinal intolerance, nephrotoxicity, and neurotoxicity. These side effects usually result in low compliance with treatment and non-adherence, and thus, the chances of developing drug resistance are elevated. More recent studies have highlighted the role of host-directed treatment, such as antioxidant and immunomodulatory agents, in enhancing treatment outcomes, drug toxicity, and host resilience in the management of TB (Jeong et al., 2022; Pires et al., 2022; Zhao et al., 2023).

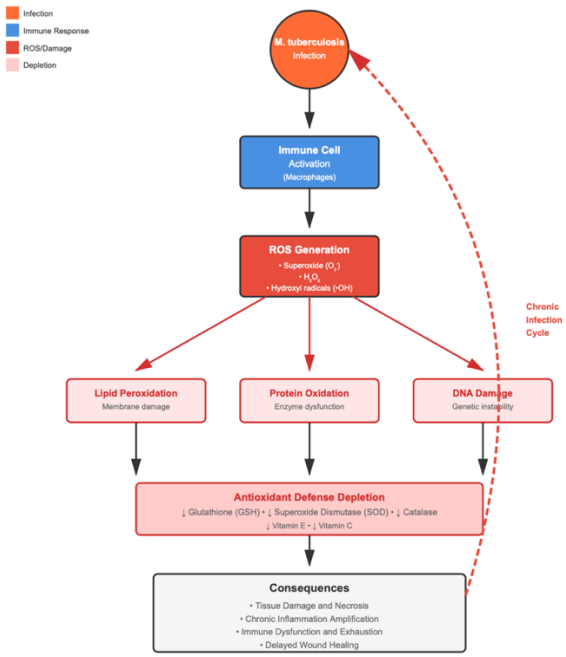

Figure 1. *Role of Oxidative Stress in Tuberculosis Pathogenesis*

Ayurveda offers a rich repository of formulations aimed at restoring tissue strength, enhancing immunity, and correcting metabolic disturbances. One of these dosage forms is sneha kalpana (oleaginous formulations) which is taking a large place in the list of dosage forms due to its ability to enhance bioavailability and tissue penetration of active constituents. Ghrita (clarified butter) is regarded as the best Sneha because it has Rasayana properties and it has the ability to carry lipophilic phytoconstituents through biological membranes and also has antioxidants and immunomodulatory constituents such as butyric acid, conjugated linoleic acid, and vitamin E (Mohammadi Hosseinabadi and Nasrollahzadeh, 2021; Munshi et al., 2021).

Nirgundi Ghrita is an Ayurvedic preparation that is referred to in Chakradatta in the part of Rajayakshma, and is customarily recommended to strengthen and energize the individuals who are weakened by chronic illnesses. Nirgundi (Vitex negundo Linn.) is a widely-growing medicinal herb which has been reported to possess antioxidant, anti-inflammatory, antimicrobial, hepatoprotective and immunomodulatory properties. The existing phytochemical and pharmacological studies have confirmed that Vitex negundo is a source of flavonoids, phenolic compounds, and other bioactive elements that eliminate free radicals and inhibit the growth of microorganisms (Wu et al., 2024; Anjali and Joshi, 2023).

The Nirgundi-Ghrita mixture is likely to increase the pharmacological activity of the formulation by increasing extraction, stability and absorption of active principles. In spite of the fact that Nirgundi and Ghrita have been individually examined to determine their antioxidant and health-promoting properties, a lack of systematic pharmaceutico-analytical and experimental research to examine Nirgundi Ghrita as a composite formulation, and specifically its antioxidant potential and antimycobacterial activity against Mycobacterium tuberculosis, exists. Such classical formulations can only be scientifically validated to fill the gap between traditional knowledge and modern evidence-based medicine (Singh et al., 2024; George & Wright, 2024).

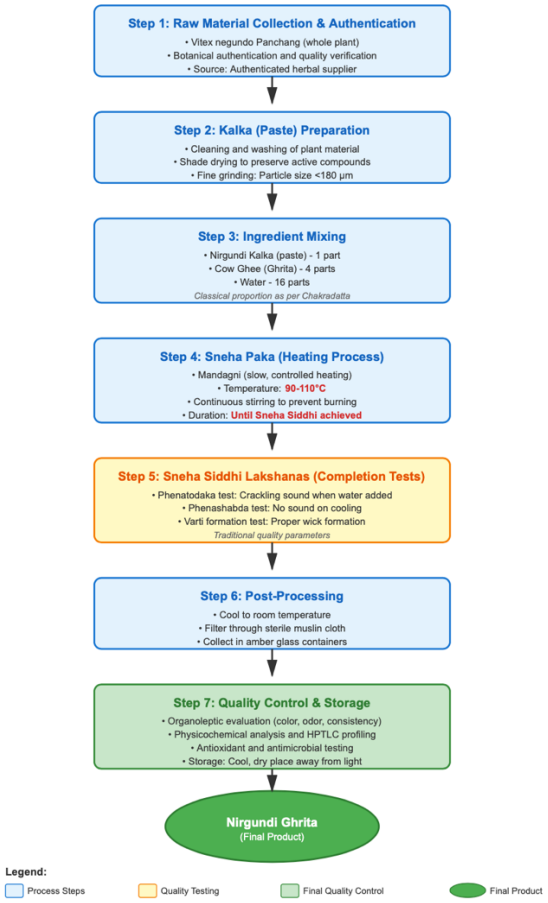

Figure 2. *Preparation Flow Chart of Nirgundi Ghrita*

Thus, the current research is aimed at conducting a pharmaceutico-analytical analysis of Nirgundi Ghrita, and then measuring its antioxidant activity and antimycobacterial capacity by means of contemporary analytical and experimental methods. The goal of this integrative methodology will be to produce scientific evidence of the traditional use of Nirgundi Ghrita in Rajayakshma and investigate its possible use as an adjunct therapy in the treatment of tuberculosis (Almarhoon et al., 2024; Bhargava et al., 2021).

1.1 Concept of Rajayakshma and Tuberculosis

In Ayurveda, **Rajayakshma** is described as a chronic, debilitating disease resulting from progressive depletion of body tissues and impairment of metabolic and immune functions. Classical literature stresses that Agni becomes deranged due to excessive physical activity, bad food habits, long term stress and repression of natural urges, which causes insufficient nourishment of Dhatus. This gradual Dhatu Kshaya influences Rasa, Rakta, Mamsa, Meda, and Shukra, which eventually leads to the depletion of Ojas, the substance that provides immunity and vitality, and thus makes the person vulnerable to chronic infectious diseases (Charak Samhita; Chakradatta).

Pulmonary tuberculosis is similar to Ayurvedic description of Rajayakshma in chronicity, weight loss, tissue wasting, cough, fatigue, and immunosuppression. Clinical effects of tuberculosis like progressive emaciation, weakness, loss of appetite and frequent infections are consistent with the Ayurvedic concept of Dhatu Kshaya and Oja Kshaya. Such conceptual similarity offers a good point of correlating Rajayakshma to pulmonary tuberculosis and studying the classical Ayurvedic formulations to support the condition (Sandhu, 2011; Bhargava et al., 2021).

Pathophysiologically, both Rajayakshma and tuberculosis are characterized by metabolic imbalance, tissue regeneration and host defence mechanisms. Chronic inflammation and chronic infection in tuberculosis cause augmented catabolism and oxidative stress, which worsens tissue damage and immune exhaustion. All these pathological processes can be linked to Ayurvedic definitions of impaired Dhatvagni and gradual depletion of Bala and Ojas, which makes integrative strategies of nourishing the tissues and restoring the immune system relevant (Naidoo et al., 2021; Qi et al., 2020).

1.2 Tuberculosis: Modern Perspective

Tuberculosis is one of the most prominent infectious morbidity and mortality causes in the world, especially in low and middle-income nations. Although standardized anti-tubercular therapy is available, the disease remains a major issue to the health of the population because of late diagnosis, long treatment process, and socioeconomic status. Global estimates demonstrate that millions of new TB cases are reported each year, and pulmonary tuberculosis is the most widespread and contagious type of the disease (Sandhu, 2011; Bhargava et al., 2023).

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis has also rendered the disease management more complex. The emergence of resistance to the first line drugs like isoniazid and rifampicin has been so severe that it has decreased the rate of success of treatment and it has led to the use of second line drugs which are less effective, more toxic and costly. The genetic and transcriptional adaptation of Mycobacterium tuberculosis is a major barrier to the eradication of TB since it causes drug resistance and survival in the host cells (Miotto et al., 2022; Rodriguez et al., 2022).

The use of anti-tubercular drugs though essential in the control of the disease is often linked to adverse effects like hepatotoxicity, nephrotoxicity, gastrointestinal and neurotoxicity. Such negative effects may undermine the patient compliance, which results in interruption of treatment and subsequent emergence of drug resistance. The long-term therapy has also been identified to modify

the oxidative stress biomarkers, which show the presence of higher oxidative load on the host during the treatment (Stanciulescu et al., 2021; Qi et al., 2020).

1.3 Need for Adjunct Therapy

The shortcomings of conventional anti-tubercular drugs have contributed to the increased attention given to the adjunct therapeutic method to improve the outcomes of the treatment and reduce the level of drug-related toxicity. Adjunct therapies are meant to boost the immune responses of the host, reduce oxidative damage, and tissue repair without affecting the antimicrobial activity of standard medications. Such approaches are particularly relevant to tuberculosis in chronic infections, where inflammation and oxidative stress have a significant contribution to the disease pathology during the long-term period (Naidoo et al., 2021; Jeong et al., 2022).

The effect of oxidative stress in tuberculosis is dual since it aids in killing the pathogen, and at the same time it leads to destruction of the host tissue when not controlled. Studies have found out that TB patients have depleted antioxidant defenses and high levels of reactive oxygen species which may worsen disease-progression and treatment-related complications. Thus, host-directed therapies and antioxidant supplementation have become potential solutions to restore the redox balance, improve immune responses, and increase patient tolerance to anti-tubercular medications (Pires et al., 2022; Zhao et al., 2023).

In the traditional medicinal systems like Ayurveda, the focus is on strengthening the host and not the pathogen. This whole-person philosophy correlates with the current ideas of host-directed therapy, which justifies the idea of assessing classical formulations with antioxidant and immunomodulatory activity as a complementary intervention in the management of tuberculosis (George & Wright, 2024; Almarhoon et al., 2024).

1.4 Sneha Kalpana in Ayurveda

Sneha Kalpana is a specialized pharmaceutical process in Ayurveda used for the preparation of oleaginous formulations such as Ghrita and Taila. These are formulations that are meant to extract, preserve and deliver lipid soluble active constituents of medicinal plants. Sneha Kalpana is an important dosage form that increases the stability of drugs, their palatability, and their therapeutic effect and is one of the most desirable dosage forms to use in internal administration (Munshi et al., 2021).

Among other Sneha Dravyas, Ghrita (clarified butter) is considered the best as it has the Rasayana action, Vata and Pitta Doshas pacifying action, and the power to nourish Dhatus and Ojas. Ghrita is said to be Yogavahi, that is, it increases the curative effect of drugs worked with it without its own qualities. This renders Ghrita a perfect vehicle of the formulations to be used in chronic and debilitating illnesses (Mohammadi Hosseinabadi & Nasrollahzadeh, 2021; Singh et al., 2024).

Modernly, Ghrita is a lipid-rich matrix that allows enhanced bioavailability of phytoconstituents by the lipid-mediated transport of biological membranes. Its anti-inflammatory and antioxidant effects are due to its content of short-chain fatty acids, conjugated linoleic acid, and fat-soluble antioxidants, which are also helpful in making it a functional therapeutic vehicle (Munshi et al., 2021; Almarhoon et al., 2024).

1.5 Nirgundi Ghrita

Nirgundi Ghrita is an Ayurvedic compound, which is mentioned in the Chakradatta as part of the chapter of Rajayakshma, and it is stated that it is used to restore strength, vitality, and general health in those with chronic wasting diseases. This formulation is given rejuvenating and disease relieving qualities in the classical text and its significance in improving Bala and Dhatu Kshaya (Chakradatta) reversal is emphasized.

Nirgundi (Vitex negundo Linn.) is yet another widely used medicinal plant which is known to possess antioxidant, anti-inflammatory, analgesic, hepatoprotective, and antimicrobial properties. The recent analytical studies have identified a large number of bioactive compounds in the Vitex negundo leaves including flavonoids and phenolic compounds that possess high free radical scavenging activity and potential antimicrobial effects (Wu et al., 2024; Anjali and Joshi, 2023).

Its therapeutic effects are likely to be increased by the processing of Nirgundi with Ghrita to obtain the active principles of the substance in an enhanced manner. Although some of the single constituents of Nirgundi Ghrita have been analyzed individually, not much scientific data assesses the formulation in totality by employing standardized pharmaceutico-analytical and experimental methods. This highlights the importance of a systematic study of Nirgundi Ghrita to confirm its classical assertion and investigate its antioxidant and antimycobacterial effects with respect to tuberculosis (Singh et al., 2024; George and Wright, 2024).

2. REVIEW OF LITERATURE

According to classical Ayurvedic texts, Rajayakshma is a disease that is a result of overworking, poor diet, repressed urges, and chronic weakness of the body tissues. According to Charaka, when Agni is impaired, Dhatus will not get

sufficient nourishment and eventually result in Oja Kshaya and predisposition to chronic diseases. The disease is defined by the presence of such symptoms like chronic cough, emaciation, fatigue, and loss of strength, which are very similar to the clinical features of pulmonary tuberculosis. The Ayurveda management approaches highlight the Rasayana therapy, Sneha Kalpana, and Balya preparations to rebuild tissue integrity and immunity (Charak Samhita).

Chakradatta gives certain recipes of the Rajayakshma management, some of which are Nirgundi Ghrita which strengthens and revives a weak person. The classical verse says that Nirgundi Ghrita consumption helps an individual with Kshaya to restore his health and vitality. This literature source emphasizes the conventional sign of Nirgundi Ghrita in chronic wasting diseases and justifies its choice in the scientific investigation in the current study (Chakradatta).

2.1 Drug Review

Nirgundi (*Vitex negundo Linn.*)

Vitex negundo is a well-documented medicinal plant in both classical and modern literature. Nirgundi is said to have Katu and Tikta Rasa, Ushna Virya, and Vata-Kapha Shamak properties, which makes it good in inflammatory, respiratory, and chronic diseases. Traditionally, the plant is used on the different parts of the body, such as cough, fever, pain, inflammation, and liver disorders, which means that it has a broad spectrum of treatment (Ahuja et al., 2015).

The antioxidant activity of Vitex negundo has been proved using the different in vitro tests conducted by modern scientific studies. Presence of flavonoid, phenolic and other secondary metabolites which are identified through phytochemical analysis have been attributed to the free radical scavenging and anti-inflammatory properties. The presence of the strong antioxidant compounds in the Nirgundi leaves has also been proved using advanced chromatographic methods that have, in turn, helped justify the use of the Nirgundi leaves in the reduction of oxidative stress-related disorders (Wu et al., 2024; Anjali and Joshi, 2023).

Besides antioxidant activity, Vitex negundo has also been known to have anti microbial and anti mycobacterial activity. Nirgundi extracts are claimed to have anti-browning effect with Gram-positive and Gram-negative bacteria and cytotoxic effect with pathogenic microorganisms. These results indicate that Nirgundi can be utilized as an antimicrobial defense mechanism and therefore it can be used in trials during the study of tuberculosis (Chowdhury et al., 2009; Kulkarni et al., 2008).

Ghrita

The Ayurvedic therapeutics places Ghrita at the center because of its Rasayana, Balya, and Yogavahi. Ghrita is a substance used in classical literature to enhance memory, digestion, immunity and nourishment of tissues. It is extensively used as an internal formula base because of its ability to penetrate into tissues with medicinal properties without being degraded (Ashtanga Sangraha).

Ghrita is nowadays rich in fat soluble vitamins, essential fatty acids and bioactive compounds such as butyric acid and conjugated linoleic acid. These ingredients have antioxidant, anti-inflammatory, and immunomodulatory effects, which make Ghrita relevant to treat chronic inflammatory and infectious diseases. It is lipophilic, which allows it to absorb and transport herbal constituents across biological membranes better (Mohammadi Hosseinabadi and Nasrollahzadeh, 2021; Munshi et al., 2021).

Pharmaceutico-analytical research on Ghrita based formulations has shown that they are stable, reproducible and can be standardized by use of modern parameters of analysis. Most recent research has highlighted the significance of physicochemical analysis and chromatographic profiling in quality control of Ghrita formulations, which is why they are scientifically validated (Singh et al., 2024).

2.2 Antioxidant Activity and Tuberculosis

Oxidative stress is a significant factor in the pathogenesis and development of tuberculosis. Mycobacterium tuberculosis infection stimulates the production of reactive oxygen species in the host defense mechanism. But prolonged oxidative stress causes damage of host tissues, inflammation aggravation and slow recovery. Patients receiving anti-tubercular therapy have been reported to have altered levels of antioxidant enzymes and elevated markers of oxidative stress (Stanciulescu et al., 2021; Qi et al., 2020).

Adjunct antioxidant therapy is suggested as a measure to counter the effects of oxidative damage, enhance immune activity and decrease drug-induced toxicity in TB patients. A number of reviews have indicated the possible use of plant-based antioxidants to reestablish redox homeostasis and host directed therapy in tuberculosis. This offers a scientific foundation of assessing Ayurvedic preparations that are rich in antioxidants when it comes to TB management (Naidoo et al., 2021; Pires et al., 2022).

2.4 Antimycobacterial Studies

Experimental research approaches in modern culture have demonstrated that it is possible to ascertain antimycobacterial activity of herbal preparations against Mycobacterium tuberculosis. Automated liquid culture systems like BacT/ALERT 3D have been extensively applied in the determination of minimum inhibitory concentrations and also in the determination of drug susceptibility. They are reproducible and valid and can be used to test conventional anti-tubercular drugs and herbal extracts (Werngren et al., 2006; Nair et al., 2016).

A number of plant-based compounds have been reported to possess potential antimycobacterial activity in vitro and therefore the investigation of natural products as adjuncts to the traditional TB treatment is viable. The importance of integrating traditional knowledge with modern experimental validation in the development of new therapeutic candidates has been mentioned in natural products and antimyobacterial drug discovery reviews (George and Wright, 2024; Almarhoon et al., 2024).

3. METHODOLOGY

The current study is planned to be a three-stage experimental research involving the combination of Ayurvedic pharmaceutical concepts and modern analytical and microbiological assessment methods. The paper is categorized into Pharmaceutical, Analytical, and Experimental stages, which allows organizing the preparation of the classical formulation Nirgundi Ghrita systematically, standardizing, and biologically testing it. This integrative design enables verifying traditional Sneha Kalpana using reproducible scientific approaches and preserving loyalty to classical sources and contemporary laboratory requirements.

3.1 Study Design

The pharmaceutical stage is concerned with the production of Nirgundi Ghrita with the help of certified raw materials and traditional processing procedures. The analytical step will entail the physicochemical characterization and antioxidant analysis of the formulation prepared, whereas the experimental step will focus on testing the antimycobacterial activity against Mycobacterium tuberculosis using an automated liquid culture system. Such a multi-phase strategy is needed to determine quality, efficacy, and translational relevance of Ayurvedic preparations to be used in chronic infectious diseases (George & Wright, 2024; Almarhoon et al., 2024).

3.2 Study Setting

Pharmaceutical preparation of Nirgundi Ghrita will be done at the Department of RasaShastra and Bhaishajya Kalpana of Mahatma Gandhi Ayurved College, Hospital and Research Centre (MGACHRC), DMIMS, Wardha. Analytical assessment will be conducted in a certified analytical lab that is a part of Datta Meghe Institute of Higher Education and Research (DMIMS), Wardha. The antimycobacterial experimental research will be carried out at the Central India Institute of Medical Sciences (CIIMS), Nagpur, where the biosafety systems and automated liquid culture systems necessary to conduct the Mycobacterium tuberculosis research are available.

3.3 Duration of Study

The overall time of the study will be 18 months, comprising of all the stages such as literature review, acquisition and validation of raw materials, pharmaceutical preparation, analytical testing, experimental testing, data gathering, statistical analysis and documentation. This time is deemed sufficient to allow the correct implementation of every stage and a methodological rigor and reproducibility of the results.

3.4 Pharmaceutical Study

Collection and Authentication

Fresh leaves and Panchang of Nirgundi (Vitex negundo Linn.) will be taken as a medicinal plant source and verified by the specialists of the Department of Dravyaguna, MGACHRC, Wardha. Pharmacopeial quality Cow ghee (Ghrita) will be sourced at a reputable source. The quality and authentication of the raw materials is essential to guarantee reproducibility and validity of the pharmaceutical results as changes in the quality of the raw drugs may have an important impact on the efficacy of the formulations (Ahuja et al., 2015; Singh et al., 2024).

Preparation of Nirgundi Ghrita

Nirgundi Ghrita will be made as per classical Sneha Kalpana procedures as stated in Ayurvedic books. The preparation will be done by heating Ghrita with Nirgundi Panchang Swarasa and Nirgundi Kalka under controlled heating conditions until the Sneha Siddhi Lakshana are attained. The process of preparation will be closely observed so that there is no uneven heating, no unstirred parts and also the active constituents are not burnt or deteriorated. The end product will be filtered and preserved in airtight containers in the appropriate conditions (Chakradatta; Munshi et al., 2021).

Sneha Siddhi Lakshana

The finish of Sneha Paka will be checked on the basis of classical signs like proper consistency of Kalka, lack of moisture, typical aroma, color and lack of crackling sound when exposed to flame. These are conventional quality indicators that are necessary to guarantee proper processing

and therapeutic efficacy of Ghrita-based preparations (Ashtanga Sangraha).

3.5 Analytical Study

Organoleptic Evaluation

Organoleptic analysis of the ready Nirgundi Ghrita will be conducted through evaluating the parameters of color, odor, taste, and consistency. These features give initial data regarding the quality of formulation, acceptability, and stability and act as control indicators of batch to batch consistency.

Physico-Chemical Parameters

The standard laboratory tests that will be used to determine the physicochemical analysis of Nirgundi Ghrita include acid value, saponification value, iodine value and loss on drying. The parameters aid in determining purity, stability, level of unsaturation and moisture content of the formulation. The chromatographic fingerprint will be produced with the help of high-performance thin-layer chromatography (HPTLC), which will allow evaluating the presence of phytoconstituents qualitatively and guarantee the standardization of the formulation.

3.6 Antioxidant Activity

The antioxidant activity of Nirgundi Ghrita will be measured by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, which is a popular in vitro test that is used to measure the antioxidant activity. The assay is used to determine the capacity of the formulation to decrease DPPH radicals leading to a decrease in the absorbance that is proportional to the antioxidant capacity. Ascorbic acid will be taken as the standard reference compound that will be compared.

The inhibition percentage of DPPH radicals will be determined at varying concentrations of the formulation and the IC 50 value (concentration needed to inhibit half of the free radicals) will be determined by using graphical means. Such a quantitative analysis will make it possible to compare the strength of antioxidants and get an idea of whether the formulation can help reduce oxidative stress related to tuberculosis.

3.7 Experimental Study (Antimycobacterial Activity)

Nirgundi Ghrita antimycobacterial activity will be compared to the H37Rv strain of Mycobacterium tuberculosis using the BacT/ALERT 3D automated liquid culture system. Such a system allows monitoring microbial growth continuously and gives credible information to determine minimum inhibitory concentration (MIC). Controls will be in the form of standard anti-tubercular drugs to confirm experimental results (Werngren et al., 2006; Miotto et al., 2022).

MIC determination will entail subjecting the cultures of the MTB to different levels of Nirgundi Ghrita extracts and observing the growth inhibition. Also, a synergistic study will be performed to determine the synergistic effect of Nirgundi Ghrita with the standard anti-tubercular drugs, and whether the formulation has the capacity to increase the antimicrobial effect or can reduce the dose of the drugs. These studies are relevant to investigate the adjunct therapeutic potential and minimize drug-related toxicity (George & Wright, 2024; Zhao et al., 2023).

3.8 Inclusion and Exclusion Criteria

The H37Rv strain of Mycobacterium tuberculosis that will be obtained in a certified laboratory will be the inclusion criterion of the experimental study. The study will not include cultures of non-mycobacterial organisms and contaminated samples. The specificity, biosafety, and validity of antimycobacterial findings are guaranteed by strict compliance with inclusion and exclusion criteria (Miotto et al., 2022).

3.9 Data Collection

The main data will be obtained through the observations made during the preparation of pharmaceuticals, analytical tests, antioxidant assays, and antimycobacterial experiments. The secondary data will be received through classical texts of Ayurveda, peer-reviewed scientific journals, and electronic databases. It is necessary to have systematic data collection to ensure the reliability of results interpretation and documentation (Bhargava et al., 2021; George & Wright, 2024).

4. EXPECTED RESULTS AND OUTCOMES

It is believed that the current research will provide a pharmaceutically standardized compound of Nirgundi Ghrita, which will be made based on the classical principles of Sneha Kalpana and will be confirmed with the help of the modern parameters of analysis. It is expected that the development of organoleptic, physicochemical, and chromatographic profiles will offer quality benchmarks that are reproducible, formulation consistency, stability, and experimental evaluation and future translational research (Singh et al., 2024; Munshi et al., 2021).

It is expected that Nirgundi Ghrita will exhibit quantifiable antioxidant properties in confirmed in vitro tests, which indicate its ability to neutralize free radicals and reduce oxidative stress. This activity would promote the

traditional application of the formulation in chronic wasting diseases and be in line with the current evidence on the antioxidant activity of Vitex negundo and Ghrita, especially in redox imbalance related to tuberculosis (Wu et al., 2024; Naidoo et al., 2021).

The experiment is also supposed to point out antimycobacterial potentials of Nirgundi Ghrita on Mycobacterium tuberculosis under controlled experimental conditions. Preliminary evidence of its classical indication in Rajayakshma would be the growth inhibition or lower growth of bacteria, which would be a first step in creating the supportive intervention. Also, the formulation can be compatible with conventional anti-tubercular medications, which indicates the potential of adjunct therapy to improve treatment outcomes and decrease the burden of treatment (Miotto et al., 2022; George & Wright, 2024).

5. TRANSLATORY COMPONENT CONCEPTUALIZED

The translatory part of the current research is the evidence-based validation of a classical Ayurvedic preparation, which is the combination of traditional knowledge and the scientific approach of the modern world. The study will contribute to increasing the scientific acceptability and reproducibility of Nirgundi Ghrita in integrative healthcare systems by setting the pharmaceutico-analytical standards and developing experimental evidence of antioxidant and antimycobacterial potential (Singh et al., 2024; Almarhoon et al., 2024).

It is expected that the findings will give a good argument to support future in vivo research and clinical trials so that they can systematically assess the safety, efficacy, and adjunct utility of Nirgundi Ghrita in the treatment of tuberculosis. These studies can examine how it can be used to enhance patient tolerance to standard therapy, decrease oxidative stress, and assist the immune system during longer treatment cycles (Naidoo et al., 2021; Jeong et al., 2022).

The study is likely to have a positive impact on the integration of Ayurvedic formulations into the management of tuberculosis, as it should provide experimental evidence that would support the idea of a holistic, patient-centered approach to the disease, complementing the existing anti-tubercular programs and addressing the existing limitations in the treatment (George & Wright, 2024; Bhargava et al., 2021).

CONCLUSION

This study provides scientific evidence for Nirgundi Ghrita, a traditional Ayurvedic formulation mentioned in Chakradatta for treating Rajayakshma, which closely resembles tuberculosis. The research combines traditional pharmaceutical methods with modern scientific testing to validate this classical remedy. The preparation is done according to the Ayurvedic principles and the present day quality standards by conducting a comprehensive analytical testing. This guarantees that the formulation is traditional and scientifically sound. The importance of testing its antioxidant property is that TB patients experience oxidative stress, which destroys tissues and impairs immunity. In case Nirgundi Ghrita is a good antioxidant, it may be used to lessen this damage. The Mycobacterium tuberculosis antimycobacterial testing will help to establish whether this formulation can directly combat TB bacteria. The results are accurate and reliable using modern laboratory techniques such as the BacT/ALERT system. The study is relevant as the cases of TB resistant to drugs are on the rise and the traditional treatment is associated with severe side effects. Nirgundi Ghrita may act as an adjunctive treatment to decrease treatment toxicity and enhance patient outcome. More to the point, this research demonstrates how the ancient medical wisdom can be proved scientifically and applied in combination with modern medicine. The results will be useful to TB patients and spur further research to other traditional formulations which should be given a scientific study.

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