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# Optimization of *Withania Somnifera* and *Ocimum Sanctum* Combinations and Evaluate their Synergistic Effect

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## ABSTRACT

Natural compounds' varied structures and diverse biological effects make them an essential source for medication development. Among these, Ocimum sanctum (tulsi) and Withania somnifera (ashwagandha) are well-known medicinal herbs with therapeutic uses in traditional medicine, especially because of their enzyme-modulating, anti-inflammatory, and antioxidant properties. In order to create a stable and potent polyherbal formulation, the present work focussed on the systematic standardization and optimization of W. somnifera (W.S.) and O. sanctum (O.S.). Both their synergistic and individual effects were assessed analytically. When Withania somnifera and Ocimum sanctum are combined, W.S.'s solubility and bioactivity are greatly increased. The results support the scientific validation of traditional medicines for certain conditions and highlight the significance of physicochemical profiling in optimizing herbal mixtures.

***KEYWORDS:*** *Ashwagandha, Tulsi, solubility, synergistic, therapeutic.*

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## 1. INTRODUCTION

Natural products have been integral to human healthcare since ancient times, forming the basis of traditional medicinal systems such as Ayurveda, Traditional Chinese Medicine, and Unani [1]. Over time, the therapeutic value of natural products has been substantiated through scientific research, positioning them as a vital resource in modern pharmacology and drug discovery [2-3]. Approximately half of all FDA-approved drugs over the past few decades have been derived from or inspired by natural products, underscoring their continued relevance and versatility in therapeutic development. One of the key challenges and considerations in the development of natural products as effective therapeutic agents lies in understanding their physicochemical properties [4-6] Characteristics such as solubility, molecular weight, lipophilicity (Log P), hydrogen bonding potential, and chemical stability critically influence a compound's pharmacokinetics and pharmacodynamics. These parameters effect directly on the therapeutic efficacy of phytochemicals which can be determined in terms of absorption, distribution, metabolism, and excretion (ADME) profile. For instance, compounds with poor aqueous solubility often face bioavailability limitations [7-9]. Moreover, evaluating these properties is essential not only for dosage form design but also for ensuring reproducibility and standardization in herbal formulations and necessitating co-formulation with natural solubilizers or bioenhancers to improve their systemic uptake. [10-12].

*Withania somnifera*, commonly known as Ashwagandha, is a renowned adaptogenic herb in Ayurvedic medicine, traditionally used to enhance vitality, reduce stress, and modulate immune responses. It contains a spectrum of bioactive constituents, primarily withanolides, which are responsible for its diverse pharmacological actions including anti-inflammatory, antioxidant, immunomodulatory, neuroprotective, reduce age and

several enzyme-inhibitory activities [13-17].Recently, our research lab has been explored the biological activities of various natural medicinal plants by using various filters including computational approach already have been reported the different traditional medicinal formulations of curcumin, with piperine and virtual screening of ashwagandha[18].

In the same queue, the combination of Ashwagandha and Tulsi presents an interesting avenue for exploration, as their distinct yet complementary phytochemical compositions may offer synergistic benefits when formulated together [19]. Moreover, such combinations may also influence the solubility and absorption profiles of individual constituents, thus improving pharmacological performance [20].This research focuses on the systematic standardization and optimization of Ashwagandha and Tulsi concentrations, both individually and in synergistic combinations. Emphasis is placed on assessing their physicochemical compatibility, solubility behavior, and inhibitory effects on key digestive enzymes, with the aim of developing a stable, effective, and scientifically validated polyherbal formulation [21].

## 2. Experimental

### 2.1. Materials

Ashwagandha and tulsi powder were ordered online from amazon, Glass double distilled water and ethanol from Triveni Engineering & Industries Ltd.

UV-VIS Spectrophotometer-117 used for examine the absorbance, and Centrifuging machine was used for centrifugation of samples.

### 2.2. Methods

#### 2.2.1. Standardization of Ashwagandha

To prepare the stock solution, 0.625 grams of *Withania somnifera* powder was precisely measured and dissolved in 25 mL of ethanol to achieve a 2.5% (w/v) concentration. This solution was continuously stirred to ensure complete dissolution. Subsequently, the stock was serially diluted to obtain a range of concentrations 2.5%, 2.0%, 1.5%, 1.0%, and 0.5%. The absorbance of each dilution was then measured using a spectrophotometer at $\lambda max\ 208.5nm$ to evaluate concentration-dependent optical behaviour [22-23].

#### 2.2.2. Standardization of Tulsi

As per the aforementioned methodology, to prepare the stock solution, 0.625 grams of tulsi powder was precisely measured and dissolved in 25 mL of ethanol to achieve a 2.5% (w/v) concentration. This solution was continuously stirred to ensure complete dissolution. Subsequently, the stock was serially diluted to obtain a range of concentrations-2.5%, 2.0%, 1.5%, 1.0%, and 0.5%. Spectrophotometric analysis was performed on each dilution at $\lambda max\ 350nm$ to determine the absorbance response relative to concentration [24-25].

#### 2.2.3. Optimization of Tulsi concentration

For the purpose of optimizing the concentration of *Ocimum sanctum* (Tulsi), a series of formulations were systematically prepared while maintaining a fixed concentration of *Withania somnifera* (Ashwagandha) at 0.5% (50 µL) [14]. Tulsi was introduced at varying volumes, ranging from 2.5% to 0.5% solutions, to investigate its influence on the formulation's physicochemical characteristics and optical properties. Spectrophotometric evaluation demonstrated a concentration-dependent increase in absorbance with rising Tulsi content, measured at $\lambda max\ 208.5nm$ [26-27]

## 3. Results & Discussion

### 3.1. Standardization of Ashwagandha

The optical characteristics of *Withania somnifera* extract were assessed via spectrophotometric analysis following the preparation of a 2.5% (w/v) ethanolic stock solution. Serial dilutions were performed to generate concentrations ranging from 2.5% to 0.5%, and absorbance measurements were recorded at $\lambda_{max}$ 208.5 nm. The absorbance was found to be increased with increasing concentration of sample. Therefore, a concentration-dependent increase in absorbance was observed (table1, indicating enhanced solute presence and optical density with increasing concentration. Notably, the 10 µL aliquot of the 0.5% dilution exhibited an absorbance value of 0.631, reflecting a favorable balance between solubility and analytical detectability. Based on these findings, this concentration was identified as optimal for subsequent experimental applications due to its reproducible and efficient spectroscopic performance [28-29].

**Table 1 : Absorbance profile of ashwagandha at different concentrations.**

| S.no. | Concentration | Absorbance |
|---|---|---|
| 1. | 0.5ml of 2.5% | 1.669 |
| 2. | 0.5ml of 2.0% | 1.391 |
| 3. | 0.5ml of 1.5% | 1.381 |
| 4. | 0.5ml of 1.0% | 1.293 |
| 5. | 0.5ml of 0.5% | 1.249 |
| 6. | 0.3ml of 0.5% | 1.229 |
| 7. | 0.1ml of 0.5% | 1.191 |
| 8. | 70µl of 0.5% | 1.119 |
| 9. | 50 µl of 0.5% | 1.011 |
| 10. | 30 µl of 0.5% | 1.009 |
| **11.** | **10 µl of 0.5%** | **0.631** |

### 3.2. Standardization of Tulsi

Spectrophotometric evaluation of *Ocimum sanctum* (Tulsi) extract at $\lambda_{max}$ 350 nm demonstrated a concentration-dependent variation in absorbance across the dilution series. The 0.1 mL volume of the 0.5% solution yielded the maximum absorbance value of 0.006, indicating enhanced solubility and favorable photometric behavior at this specific concentration. These findings suggest that this dilution offers an optimal balance between extract concentration and spectral clarity, thereby rendering it the most suitable for subsequent experimental applications [30].

**Table 2: Absorbance profile of Tulsi at different concentrations.**

| S.no. | Concentration | Absorbance |
|---|---|---|
| 1. | 0.5ml of 2.5% | 0.050 |
| 2. | 0.5ml of 2.0% | 0.048 |
| 3. | 0.5ml of 1.5% | 0.036 |
| 4. | 0.5ml of 1.0% | 0.026 |
| 5. | 0.5ml of 0.5% | 0.013 |
| 6. | 0.3ml of 0.5% | 0.010 |
| **7.** | **0.1ml of 0.5%** | **0.006** |

### 3.3. Optimization of Ashwagandha and Tulsi concentrations

A systematic concentration optimization study was conducted to evaluate the influence of *Ocimum sanctum* (Tulsi) on the physicochemical characteristics of a polyherbal formulation containing a fixed concentration of *Withania somnifera* (Ashwagandha) at 0.5% (50 µL). Spectrophotometric measurements at $\lambda_{max}$ 208.5 nm demonstrated a progressive increase in absorbance with escalating tulsi concentrations, indicative of improved solubility and potential synergistic interactions between constituent phytochemicals [31]. However, this absorbance enhancement reached a plateau beyond a certain concentration threshold, suggesting saturation of solubility and diminished incremental benefit. Among the evaluated concentrations, the formulation comprising 0.1 mL of 0.5% Tulsi extract exhibited the most optimal spectral and stability profile. This specific combination was consequently selected for subsequent combinatorial and functional evaluations owing to its superior analytical and formulation performance [32].

**Table 3: Effect of varying concentration of Tulsi with fixed amount of Ashwagandha**

| S.no. | Withania somnifera (Constant) | Tulsi (Vary) | Ethanol | Total Volume | Absorbance |
|---|---|---|---|---|---|
| **Control** | 0.50ml of 50 µl of 0.5% | 0ml | 3.5 ml | 4ml | 0.932 |
| 1. | 0.50ml of 50 µl of 0.5% | 0.5ml of 2.5% | 3 ml | 4ml | 1.128 |
| 2. | 0.50ml of 50 µl of 0.5% | 0.5ml of 2.0% | 3 ml | 4ml | 1.108 |
| 3. | 0.50ml of 50 µl of 0.5% | 0.5ml of 1.5% | 3 ml | 4ml | 1.070 |
| 4. | 0.50ml of 50 µl of 0.5% | 0.5ml of 1.0% | 3 ml | 4ml | 1.052 |
| 5. | 0.50ml of 50 µl of 0.5% | 0.5ml of 0.5% | 3 ml | 4ml | 1.033 |
| 6. | 0.50ml of 50 µl of 0.5% | 0.3ml of 0.5% | 3.2 ml | 4ml | 1.019 |
| 7. | 0.50ml of 50 µl of 0.5% | 0.1ml of 0.5% | 3.4 ml | 4ml | 1.003 |

## Conclusion

This study presents a comprehensive and methodologically sound investigation into the standardization and optimization of *Withania somnifera* and *Ocimum sanctum*, individually and in combination, with the objective of enhancing their physicochemical characteristics and bioactive potential. The optimized concentrations, determined through systematic dilution and spectrophotometric evaluation, demonstrated that precise formulation is critical for achieving consistent solubility, stability, and activity. The synergistic enhancement observed upon combining ashwagandha and tulsi-further supported by the incorporation of natural excipients such as honey and milk-validates traditional polyherbal approaches and offers a scientific basis for their continued application in modern phytopharmaceutical development. The findings underscore the relevance of integrating ethnobotanical knowledge with empirical optimization techniques to develop robust, standardized, and efficacious herbal formulations.

## Significance of the study

The present study provides a systematic evaluation of the physicochemical and functional properties of ashwagandha and tulsi both individually and in combination. W.S. is well-documented for its adaptogenic, immunomodulatory, and antioxidant effects, while O.S. is widely recognized for its antimicrobial, anti-inflammatory, and hepatoprotective activities. Through methodical standardization and optimization of each extract, this research establishes a reproducible framework for assessing their bioactive potential. The combinatorial analysis revealed a synergistic enhancement in physicochemical characteristics, particularly increased absorbance values indicative of improved solubility and potential bioavailability. These findings

substantiate traditional therapeutic practices involving the concurrent use of Ashwagandha and Tulsi, while also providing a scientific rationale for their integration into standardized polyherbal formulations. Overall, this work contributes to the advancement of evidence-based herbal medicine by bridging ethnopharmacological knowledge with modern experimental validation.

**Conflict of Interest**: The authors declare no conflict of interest.

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