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Synergism in Solubility of Polyherbal Formulations of Garlic: Standardization and Optimization

Manisha[1], Khushboo[2], Neera Raghav[3], Heena Dahiya[4], Nitika Mor[5*]

[1,2,4,5]Department of Chemistry, Baba Mastnath University, Rohtak, Haryana, India
[3]Department of Chemistry, Kurukshetra University, Thanesar, Haryana, India
*Corresponding Author: Dr. Nitika Mor

ABSTRACT

Systems like Ayurveda, Traditional Chinese Medicine, Unani, Siddha rely on natural products as their foundation. Standardization of herbal plants should focus on reducing any potential toxicity and upholding the therapeutic efficacy, quality, and safety. Standardization involves the identification, authentication and quantification of active pharmacological constituents along with the evaluation of physicochemical parameters, to ensure quality assurance and maintain batch- to- batch consistency. The current study aims at the systematic standardization and optimization of garlic and O. sanctum to formulate a stable, pharmacodynamically active polyherbal preparation. Analytical evaluation methods were employed to assess the individual and synergistic contributions of these botanicals. It was observed that the solubility of active components derived from garlic exhibit greater when combined with tulsi due to synergistic effect. These findings provide a scientific foundation for the therapeutic importance of traditional herbal mixtures, emphasising the significance of physicochemical characterization for rational optimization of polyherbal formulations.

KEYWORDS: *Garlic, Tulsi, solubility, synergistic effect, therapeutic application.*

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1. INTRODUCTION

Natural products are backbone of traditional medical system and also serve as foundation for modern medicine as many modern drugs are isolated from natural resources. Natural products are also applied in different domains such as nutrition, food technology, cosmetics, biomaterial development and for multifarious industrial processes [1]. Besides medicine, natural products and their derivatives are widely used as food additives as spices and herbs, anti-bacterial and antioxidants in food preservation and storage. Sixty percent of all medicines, either directly or indirectly, from natural products and used extensively in pharmaceutical industries. Because they have minimum side effects or hardly have any and available very easily in the nature. The majority of the tropical population depended primarily on traditional medicinal systems particularly in the developing world [2-3].

One of the tasks in drug discovery is to identify target proteins of natural products prior to their application as medicines to avoid deleterious effects, for example, the interaction between natural products and proteins can be elucidated using affinity chromatography. Different drugs have been researched considering their structural properties, action mechanisms, cellular uptakes and other biological properties and have been approved by FDA (Food and Drug Administration). Natural products are the source of high-quality leads, further research on these natural products can help to clarify understanding of biological processes, their mechanism of actions which can lead to new drug development [4-5]. Plants represent the most plentiful sources of natural medicine because of their structural and chemical diversity as well as the biodiversity of their components.

The physicochemical properties of natural product–based therapeutics are essential to their development [6]. These class properties alter the ADME profiles of phytoconstituents, leading to direct dependence on

their therapeutic action. Despite the numerous predicted advantages, poor aqueous solubility, for example, still constitutes a critical barrier to bioavailability of many phytochemicals [7,8]. However, such systematic characterisation of these properties is a prerequisite for rational dosage-form design, quality control and standardisation of herbal formulations.

Article highlights that even in the small intestine, bioavailability is often limited, and in many cases co-formulation with naturally occurring solubilizing agents, such as bile salts, phospholipids or bioenhancers is necessary to achieve systemic levels of exposure [9–11].

Garlic (Allium sativum) is a medicinal plant widely used in folk and complementary medicine because of the wide spectrum of its pharmacological activities. The therapeutic potential of garlic is mainly ascribed to its rich variety of organosulfur compounds like allicin, ajoene, diallyl sulfide and diallyl disulfide that are linked with antimicrobial, antioxidant, anti-inflammatory, anticancer, cardioprotective, immunomodulatory, and enzyme-inhibitory activities [12–14]. Over the last few years, our research team has studied the biological activities of several medicinal plants using combined screening strategies, including computational tools. However, exploratory studies have reported tablets containing traditional turmeric formulations with piperine, as well as the analytical virtual screening of bioactive phytoconstituents, ultimately sourced from garlic [15-17].

Herein, garlic co-administration with *Ocimum sanctum* (Tulsi) may serve as a potential avenue to improve therapeutic potency. The disparate but complementary phytochemical profiles of these two medicinal plants may yield additive biological responses due to the ideal synergistic nature of their co-administration [18]. In addition, these polyherbal preparations may alter the solubility and absorption characteristics of individual components, thereby rendering them more favourable and improving the therapeutic profile [19-20].

The present study was performed by systematic standardisation of concentration of individual and combinatory treatment of garlic and Tulsi to determine the optimum concentrations.

2. Experimental

2.1. Materials

Commercially available Ocimum sanctum powder and garlic powder (Amazon) were used. The analytical-grade glass double distilled water and ethanol were provided by Triveni Engineering & Industries Ltd. UV-Visible analyses were conducted using a UV-Visible spectrophotometer and laboratory centrifuge for sample separation.

2.2. Methods

2.2.1. Standardization of garlic

Preparation of stock solution 0.625 g of garlic powder was accurately weighed into a 25 ml ethanol to make a 2.5%(w/v) solution. The mixture was stirred to the maximum extent until completely dissolved. Next, serial dilutions were performed to obtain solutions of 2.5%, 2.0%, 1.5%, 1.0%, and 0.5%. For assessment of concentration-dependent optical properties of garlic, a UV-Visible spectrophotometer was used to measure the absorbance of each dilution at 220 nm.

2.2.2. Standardization of Tulsi

Based on those methods, 0.625 g of tulsi powder (dried tulsi leaves were ground into fine powder) was weighed on analytical balance and 25 ml of ethanol was added to obtain 2.5% (w/v) stock solution. To achieve complete solubilization of the extract the solution was mixed several times. Serial dilutions were then prepared to give 2.5%, 2.0%, 1.5%, 1.0% and 0.5%. The absorbance at maximum wavelength (λ max) of 350 nm of each dilution was measured with UV-Visible spectrophotometer (Bio spectrophotometer, Japan) to comparatively evaluate the concentration-dependent spectrophotometric behaviour.

2.2.3. Optimization of Tulsi concentration

Formulations were prepared for optimization, to get optimum concentration of *Ocimum sanctum* (Tulsi), keeping the concentration of *Allium sativum* (garlic) constant at 0.1 ml of 0.5%. To assess the effect on the physicochemical properties and optical characteristics of the formulations, Tulsi was incorporated at 2.5% and 0.5% solution concentrations. Spectrophotometric analysis showed an increase in absorbance with a quantitative increase in tulsi content at the maximum wavelength (λ max) of 220 nm [21-22].

3. Result and discussion

3.1. Standardization of garlic

UV-Visible spectrophotometric characterisation of the prepared 2.5% (w/v) ethanolic stock solution of *Allium sativum* (garlic) extract was carried out to check the optical behaviour. Series of dilutions were prepared to generate concentration ranging from 0.5% to 2.5%, and absorption was recorded at the maximum wavelength (λ max) of 220 nm. In other words, with an increase of extracts concentration the absorbance which is an optical response depending on solute density at higher concentrations more was observed in table1. For instance, 0.1ml of 0.5% dilution showed the absorbance value at 0.738, which is sufficient for photometric detection and confirms

appropriate trade-off between solubility and sensitivity. On the basis of these observations and for the reproducible, predictable spectroscopic properties, this concentration was chosen for further experimental work [23-24].

Table 1: Absorbance profile of garlic at various concentrations

S. no.	Concentration	Absorbance
1.	**0.1ml of 0.5%**	**0.738**
2.	0.3ml of 0.5%	0.812
3.	0.5ml of 0.5%	0.927
4.	0.5ml of 1.0%	1.103
5.	0.5ml of 1.5%	1.270
6.	0.5ml of 2.0%	1.405
7.	0.5ml of 2.5%	1.601

3.2. Standardization of Tulsi

This determined the absorbance of Tulsi extract along the series of dilutions, measured by UV-Visible spectrophotometry at maximum wavelength (λ max) is 350nm depicted in table2. From the series of dilutions, 0.1 ml of 0.5% solution showed better absorbance at 0.006 value this shows that compounds become more soluble into water thus allowing better photometric characteristics at this strength. Observations indicates that this dilution provided an optimal compromise between extract volume versus spectra resolution from that extract and thus can be amenable to deeper experimental interrogation [25-26].

Table 2: Absorbance profile of Tulsi at various concentrations

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

3.3. Optimization of Garlic and Tulsi concentrations

A systematic concentration optimization study was carried out to assess the effect of *Ocimum sanctum* (Tulsi) on the physicochemical properties of a polyherbal formulation containing a fixed concentration of garlic at 0.1 ml of 0.5% presented in table3. The absorbance increased progressively with increasing concentration of tulsi (at λ_{max} 220 nm), presumably due to the increasing solubility of contributory phytochemicals and synergistic effect [27]. However, the increase in absorbance stabilized after a specific concentration limit, indicating that solubility had reached a saturation point and further increases provided minimal additional benefit. Among all the tested ratios 0.5% formulation containing volume of 0.1 ml of tulsi extract exhibited best spectroscopic properties as well as good stability. Therefore, this optimised mixture was selected for subsequent combinatorial and functional analysis due to its improved analytical performance and stability in formulation [28].

Table 3: Effect of change amount of Tulsi with constant value of Garlic

S. no.	Garlic (Constant)	Tulsi (Vary)	Ethanol	Total Volume	Absorbance
Control	0.50 ml of 0.1ml of 0.5%	0 ml	3.5 ml	4ml	0.738
1.	**0.50 ml of 0.1 ml of 0.5%**	**0.1ml of 0.5%**	**3.4 ml**	**4ml**	**0.988**
2.	0.50 ml of 0.1 ml of 0.5%	0.3ml of 0.5%	3.2 ml	4ml	1.053
3.	0.50 ml of 0.1 ml of 0.5%	0.5ml of 0.5%	3 ml	4ml	1.103
4.	0.50 ml of 0.1 ml of 0.5%	0.5ml of 1.0%	3 ml	4ml	1.174
5.	0.50 ml of 0.1 ml of 0.5%	0.5ml of 1.5%	3 ml	4ml	1.259
6.	0.50 ml of 0.1 ml of 0.5%	0.5ml of 2.0%	3 ml	4ml	1.366
7.	0.50 ml of 0.1 ml of 0.5%	0.5ml of 2.5%	3 ml	4ml	1.399

Conclusion

The present study attempts to standardise and optimise Allium sativum (Garlic) and Ocimum sanctum (Tulsi), individually as well as in combination, for the development of physicochemical profiles and bioactive efficacy. This work greatly illustrates how custom formulations increase application usability with regard to solubility, stability, and desired biological activity that can be achieved through controlled dilutions and spectrophotometric characterizations. The results of the findings showed a pronounced additive effect on the physicochemical properties, with higher absorbance values, especially supporting higher solubility and thus potentially increasing bioavailability. Here, we provide scientific validation of the traditional practice of using garlic and tulsi together and offer a rationale for their inclusion in generic multi-herbal formulations.

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