

A Review On Polyherbal Formulations For Immune Enhancement: Phytochemical Standardization, Antioxidant Potential, And Immunomodulatory Activity Of Traditional Rasayana Herbs

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ABSTRACT

Despite the slogan “health for all” announced by the WHO, the world is still struggling to deal with the issues associated with imbalance immunity. Advances in drug discovery and innovative research have flourished until now, but research promoting natural drugs is less than 10%, as compared to synthetic entities. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defense mechanisms have to be activated under the conditions of impaired immune responsiveness. The major advantage of herbal medicine is without causing any adverse effects and is of less expensive, when compared to synthetic drugs.

India is one of the richest nations in terms of biodiversity, as it has more than 45,000 plant species, and it is estimated that about 1500-2000 species have medicinal use, almost 20,000 medicinal plants have been recorded and codified. The herbs possess immunomodulatory, free radical scavenging, antioxidant, anti-inflammatory, anti-infective, adaptogenic, hepatoprotectant, and neuroprotectant activity thereby promoting immune enhancement. In this article, based on ancient literature and clinical studies the evidence-based outcome of Rasayana herbs useful as an antioxidant and for Immunomodulation has been summarized. In addition, an attempt is being made for the importance of Phytochemical and Pharmacological standardization of Rasayana herbs which are the fundamental needs for scientists and clinicians to develop a meaningful Rejuvenator Rasayana polyherbal formulation to serve promising health benefits to society.

Ayurveda is the branch of knowledge or “science” (Veda) of life (Ayus). Rasayana therapy is used for preventive, promotive and curative action, to reduce the effect of ageing and improve the quality of life. Rasayana is an auxiliary discipline which deals with a series of specialized herbal preparations, food articles and lifestyle which must be acquired and practiced for the attainment of the best physical and psychological health. Rasayana herbs show a beneficial effect on immune function,- a dose-dependent increase in the primary and secondary humoral immune responses, and also enhance intracellular bacterial killing capacity by augmenting the release of reactive oxygen from macrophages. Numerous rasayanans are known to increase the resistance of the body against common infections, and they have shown encouraging results as adjuvants to chemotherapy and radiation therapy in cancer. Over the last decade, the downfall in lifestyle maintenance is progressing towards immune failure! The post-pandemic alertness, antibiotic resistance, and toxic profile of synthetic immunostimulators have revived scientific attention towards plant-based immunostimulants. In this review, pertinent research on the development and evaluation of polyherbal immunobooster dispersible tablets is presented. The evidence of the Rasayana effect of each individual herb amongst the 6 selected herbs- Emblica officinalis, Tinospora cordifolia, Withania somnifera, Ocimum sanctum, Curcuma longa, and Zingiber officinale are discussed in detail. The key findings of polyherbal dispersible tablets evaluation as per United States Pharmacopoeia - official monographs suggest that physicochemical properties and dispersion-time meet standard criteria. Flavonoids, phenolics, tannins, saponins, and alkaloids are evident in the quantity standards. DPPH activity in percentage inhibition is always greater than 78. The phagocytic index in in-vitro immunomodulatory activity is significantly higher than control. The Carr. The carr index and The Hausner ratio reveal good powder-flow. IN addition, the results of a 30-day stability study of dispersible tablets at 40°C/ 75%RH showed insignificant changes in pre-and post-compression and phytochemical parameters.

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Collectively, all the evidence supports the rational use and marketing of polyherbal immunobooster dispersible tablets as a safe and effective stimulant of the immune system as compared to synthetic immunotherapy!

Keywords: Polyherbal formulation, immunomodulation, Rasayana, DPPH antioxidant assay, dispersible tablet, *Withania somnifera*, *Emblica officinalis*, *Tinospora cordifolia*, phytochemical standardization, macrophage activation.

INTRODUCTION

Immunity is the most important system of the body. It is categorized as first-line, non-specific, and at times-specific response to pathogens to protect the body from any antigenic insult without creating damage to host tissue[1,6]. There are two types of immunity: innate/ non-specific immunity which involves the action of macrophages, neutrophils, natural killer cells, and complement system; and adaptive/acquired/specific immunity which involves B-lymphocytes, T-lymphocytes, memory cells, and immunoglobulins [9].

Recent crisis of global disease burden has given unforgettable pace to the need for preventive immune-stimulant in human life. The burden of emerging and re-emerging viral diseases, chronic stress, erratic lifestyles, nutritional deficiency, and the development of antibiotic resistance in microbes has drastically deteriorated the immune potential in recent times [3,14]. This demands the maintenance of innate immunity rather than treatment after the establishment of infection in humans. Ayurveda mentions herbs for the improvement of the immune system under the category of Rasayana drugs.

Rasayana drugs are stated to have tissue promotive action which complete nourishment and responsible for longevity, improved memory, retentions, intelligence, youthfulness, preeminence in complexion and luster, optimum development of physique and senseorgans, mastery over speech, respectability and brilliance [12]. In the classical Sanskrit literature, the word Rasayana has been described as a means of functional nourishment and one obtaining such a state is not affected by any disease. Pharmacological evaluation of Rasayana drugs has proved many of these claims [12,19,28]. Herbal drug development in the form of proper dosage formulation that can be standardized is a challenging task. Since the raw material for plant-based medicines is derived from a wide variety of

geographical sources, standardization of finished medicine or the product is of utmost importance. Dispersible tablets are an attractive dosage form for developing and marketing polyherbal immunobooster formulations since they are easy to administer in pediatric and geriatric patients and do not require water for dissolution so dissolution takes place quickly in the neutral condition of the oral cavity [13,16].

In this review, the development of dispersible polyherbal immunobooster tablet- phytochemical standardization, antioxidant, immunomodulatory evaluation, and stability study evidence has been summarized for a total of 6 Rasayana botanicals.

BOTANICAL PROFILE OF IMPORTANT IMMUNOMODULATORY HERBS

1. *Emblica officinalis* (Amla)

Amla is the richest natural source of vitamin C [8,11]. Amla has reportedly 20 times higher vitamin C content than citrus fruits, in addition to emblicanin-A, pedunculagin, punigluconin and emblicanin-B. It is considered to be the best among

rejuvenative herbs in Ayurveda, and is useful as vayasthapana (arresting age), medhya (general tonic and memory enhancer) and for immunomodulation. *Emblica officinalis* enhances natural killer cell activity and phagocytosis by macrophages as well as haematopoiesis, as evidenced by increased IgM and IgG titer in in vivo studies. It is also found to be hepatoprotectant.[4,5]



2. *Tinospora cordifolia* (Giloy / Guduchi)

Giloy is highly regarded by present immunologists as it is well proven to act on the innate immune system (Nikhil Sharma, Yogendra Krishna MS et al. studied the immunomodulatory activity of Guduchi in patients with Dengue and allergic rhinitis. The results suggest improvement in platelet count and the neutrophil to lymphocyte ratio. Stress is widely considered to be immunosuppressive. Giloy is adaptogen and immunostimulating to protect immune balance in the body during a stressful situation. During infection, this activity is beneficial in promoting recovery while in normal health it may provide resistance to system stress. Both these roles support its recognition as a Tridoshanashak Rasayana dravya in Ayurveda classical text. [15,19]



3. *Withania somnifera* (Ashwagandha)

Over thousands of years (and more so in the past two decades of exhaustive research), Ashwagandha has been excessively explored for its immunopotentiator, adaptogenic, antioxidant, antistressor, and anabolic effect. It is rejuvenator in classical Ayurvedic text and promising clinical studies are available on its immunomodulatory and stress-mitigating activity. In clinical trials on elderly ill-appearing persons, Ashwagandha supplementation has led to a significant increase in IgG subclasses, especially IgG2 levels, and overall immunity perks up. [12,

Withanolides are the steroidal alkaloids responsible for medicinal activity in Ashwagandha [1,8]. In clinical trials on adults under extreme psychological stress and consequent low level of immunity, Ashwagandha supplementation has led to an augmented T cell count (notably an increased CD4⁺ T cell count) and increased and increased serum immunoglobulin [2,10].



4. *Ocimum sanctum* (Tulsi)

Tulsi has been regarded as universal protector of health in Indian tradition. It is marginally immunomodulator in action and enriches the phytochemical profile of immunostimulatory polyherbal formulation with its eugenol, and antioxidants.

In clinical trial on healthy persons, Tulsi has promoted antibody production, macrophage migration index and IFN-gamma production. [3,18]

The abundant flavonoid apigenin in Tulsi is responsible for its measurable suppression of inflammation through a marked inhibition of pro-inflammatory prostaglandin E2 via down-regulation of the cox-2 enzyme, without impeding physiological levels of inflammation required for protection against infection as evidenced by amelioration of clinical symptoms of fever induction and systemic-inflammation ("overall symptomatic score") despite simultaneous increase in IFN-gamma and rise of body temperature. [31] 2.5. Curcuma longa (Haldi) and Zingiber officinale (Ginger)



Perhaps the most famous popular and most studied phytochemical ever, the bright yellow pigment and key pharmacophore of turmeric, curcumin, is an all-around anti-inflammatory and anti-oxidant agent [13,25]. In support of the extensive body of evidences for these actions, curcumin is also able to scatter cytokine production and mesage regulated inflammation by immune cells by inhibiting master transcription factor activation of anti-bacterial peptides in the immune response, while at the same regulating protein that orchestrates excessive tissue-destructive inflammation. It is proven to stimulate T-cell proliferation and macrophage activation. Turmeric is always described as a critical immunomodulation in classical Ayurveda-literature.

Ginger compliments turmeric with its gingerol and shogaol content, responsible for antioand antimicrobial activity. In pre-clinical studies, ginger extracts have been shown to proliferate T-c stimulate macrophages. TNF-alpha is a target in limiting the destructive potential of excessive inflammation in diseases like arthritis, and ginger has been mapped to modulate tissue destruction through this pathway in preclinical studies.[20,24]

RATIONALE FOR POLYHERBAL COMBINATION

The ancient Ayurvedic system of pharmacy puts forth the concept of Anupana, which means that the action of each herb is potentiated when administered in proper combination with others. Pharmacologically, such an effect is termed as synergism, where two or more phytochemically diverse herbs, acting through different molecular mechanisms but on the same immunological pathway, potentiate the effect that would be achieved if the individual ingredients were administered alone [9,16]. In case of immunity, a polyherbal combination confers several apparent benefits. *Emblica officinalis* forms the core antioxidant scaffold and provides humoral immunity. *Tinospora cordifolia* and *Withania somnifera* provide direct macrophage activation and lymphocyte proliferation. *Ocimum sanctum* extends antiviral and anti-inflammatory support. *Curcuma longa* and

Zingiber officinale counter oxidative burden and cytokine imbalance [6,13]. Thus, the entire range of immune response, from barrier immunity to memory cells, is addressed using a lower dose of each ingredient, minimizing the risk of dose-dependent side effects. In addition, the polyherbal combination promotes mutually increased bioavailability. Piperine like alkaloids present in some of the above herbs inhibit p-Glycoprotein mediated efflux, therefore enhancing the mean residence time of curcumin, withanolides and other poorly available components in the intestinal mucosal cells.

This pharmacokinetic synergism is an important practical consideration in formulation design and helps explain why many traditional polyherbal preparations demonstrate clinical efficacy at doses where isolated phytochemicals would be pharmacologically inert [2,9].

FORMULATION DESIGN AND PHARMACEUTICAL DEVELOPMENT

1. Selection of Dosage Form

Dispersible tablets offer several important advantages over decoctions, powders and churna dosage forms for herbal immunoboosters. Dosing is accurate, shelf-life is prolonged due to minimal moisture interaction, and the main disadvantage of storage-induced oxidation of thermolabile phytoconstituents is eliminated [17,26]. Dispersible tablets rapidly disintegrate in a small volume of water, usually within three minutes, to form a consistent suspension that is easier to swallow for geriatric and pediatric patients who are particularly at risk of immune suppression. If, after sieving herbal extracts through mesh #40, flow properties are sufficient, direct compression is the preferred method for manufacturing as it precludes the use of heat and moisture during wet granulation, which can destroy heat labile phenolics, flavonoids and essential oil components that contribute strongly to immunopharmacological activity [20,24].

2. Excipient Selection and Rationale

Excipient	Category	Functional Role in Formulation
Microcrystalline Cellulose (MCC)	Diluent / Binder	Provides compressibility; enhances tablet mechanical strength

Crospovidone / Sodium Starch Glycolate	Super-disintegrant	Rapid water uptake and swelling ensuring ≤ 3 min disintegration
Mannitol	Sweetener / Diluent	Improves palatability; provides cool mouthfeel; low hygroscopicity
Magnesium Stearate	Lubricant	Reduces die-wall friction; prevents tablet sticking
Talc / Aerosil	Glidant	Improves powder flow through hopper and die feeder
Citric Acid + Sodium Bicarbonate	Effervescent Pair (optional)	Accelerates dispersion through CO ₂ liberation in water
Natural Flavor (Lemon/Orange)	Taste Masking	Attenuates residual bitter-astringent notes of tannin-rich extracts

The use of crospovidone as the main super-disintegrant is justified by its more rapid water-uptake compared to croscarmellose sodium in the presence of tannins, which tend to interact with cellulose and slow down water absorption [2,9,13]. 4.3 Approach to Formulation Optimization

Several trial batches were manufactured with variations in composition of superdisintegrants (5-8%), magnesium stearate (0.5-1.5%) and herbal extract to diluents ratio. The optimized batch was selected based on hardness, friability, disintegration time, wetting time, water absorption ratio and palatability. Average tablet weight is 400-500 mg and disintegration time of the optimized formulation is not

more than three minutes in 200 mL water at room temperature.

EVALUATION PARAMETERS AND RESULTS

1. Physicochemical and Organoleptic Evaluation

Preliminary physicochemical testing of the formulation blend gave the following results, which are as expected for tannin and phenolic rich extracts.

Organoleptic evaluation revealed a light to dark brown appearance, aromatic odor mainly due to Amla and Ashwagandha components, and a bitterastringent aftertaste which was reduced by the added sweetener and flavor. [5,17] Physicochemical data are displayed in the table below:

Parameter	Observed Result	Reference Standard
Loss on Drying (Moisture Content)	2.78%	NMT 5% (WHO/IP)
Total Ash	5.12%	NMT 6% (Ayurvedic Pharmacopoeia)
Acid-Insoluble Ash	1.45%	NMT 2%
Water-Soluble Extractive Value	37.24%	As per monograph
Alcohol-Soluble Extractive Value	24.62%	As per monograph
pH of 1% Dispersion	5.72 $\pm$ 0.02	5–7 (acceptable for oral use)

Low moisture content (2.78%) is an important indication of manufacturability and storability of the formulation, since excessive moisture breeds growth of microbes as well resulting in hydrolysis of glycosidic bonds and esters that are phytochemically rampant in the formulation ingredients [28,29].

2. Phytochemical Screening

Phytochemical screening of the combined extract revealed the presence of all major classes of immunologically active secondary metabolites. Dragendorff and Mayer tests were positive for alkaloids, the Shinoda test indicated the presence of

flavonoids, Ferric Chloride reaction indicated the presence of both tannins and phenolic compounds, Froth test confirmed the presence of saponins and the

Keller-Killiani test was positive for cardiac glycosides. Results are depicted in the following table:

Phytochemical Class	Detection Method	Result
Alkaloids	Dragendorff & Mayer Tests	Positive (+)
Flavonoids	Shinoda Test	Positive (+)
Tannins	Ferric Chloride Test	Positive (+)
Phenolic Compounds	Ferric Chloride Test	Positive (+)
Saponins	Froth Test	Positive (+)
Glycosides	Keller-Killiani Test	Positive (+)
Terpenoids	Liebermann-Burchard Test	Positive (+)

The confirmed presence of flavonoids and phenolic compounds is especially significant, since these compounds regulate immunity by scavenging reactive oxygen species that inhibit lymphocyte proliferation, by down regulating pro-inflammatory mediators and by initiating a cascade of intracellular immune-

signaling events [11,19]. Saponins have been independently indicated in enhancing phagocytic activity of macrophages and stimulating mucosal immunity.

3. Powder Flow and Compressibility

Flow Parameter	Measured Value	Interpretation
Angle of Repose	28.5°	Excellent flow (< 30°)
Bulk Density	0.45 g/ml	Good compressibility
Tapped Density	0.52 g/ml	—
Carr's Compressibility Index	13.46%	Good (< 15%)
Hausner Ratio	1.15	Good flow (< 1.25)

Flow properties within pharmacopoeially acceptable limits indicate that the blend can be compressed directly without the risk of dose variation. A compressibility index (Carr's index) below 15% and degree of flowability (Hausner ratio) below 1.25 are indicators of favorable balance between inter-particle cohesive and repulsive forces [18] and these observations jointly validate the suitability of direct compression approach to formulation manufacture.

4. Antioxidant Activity: DPPH Free Radical Scavenging

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method is the most commonly cited procedure for determining anti-oxidant activity of herbal extracts, and may be considered as an indicator of immune protection. The following results were obtained for the formulation under investigation

Sample	% DPPH Inhibition	Remark
Polyherbal Formulation Extract	78.56%	High antioxidant activity
Standard – Ascorbic Acid (Vitamin C)	92.14%	Reference standard

The value is comparable to data reported for other polyherbal formulations with immunomodulatory activity. There is a considerable difference in activity between the formulation and the vitamin C reference

standard, but this is due to curcuminoids from *Curcuma longa* and ascorbic acid equivalents from *Embllica officinalis* are responsible for the majority of observed anti-oxidant activity [4,13,23]

Immunologically speaking, high anti-oxidant activity prevents oxidative damage to cellular and organelle lipid membranes of immune cells, fragmentation of lymphocyte DNA and free radical-induced cytokine imbalance which is the main cause for reduced acquired immunity in chronic metabolic disorders and cancers [5,11]

5. Immunomodulatory Activity: Carbon Clearance Assay

Group	Phagocytic Index	Change vs. Control
Negative Control	1.00	—
Polyherbal Formulation Extract	1.65 ± 0.05	+65% above control

This increase in phagocytic index by 65% compared to control is mediated mainly by well documented activity of withanolides from Ashwagandha, tinosporin from Guduchi and apiin from Tulsi [8,19,27] Macrophage activation is the first step in an adequate and appropriate immune response to pathogenic invasion, and boosting phagocytic activity has long term effects on acquired immunity such as increased circulating T and B lymphocytes and production of memory cells.

6. Microbial Safety and Accelerated Stability

Microbial testing (data not shown) confirmed that the formulation was free from entero-pathogens including *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*, and that both total aerobic microbial count and yeast & mould count were within the WHO-stipulated maximum limits for herbal products intended for oral consumption [17,29] This corroborates the use of hygienic processing and packaging conditions.

Over 30 days of storage in open Petri plates at 40 ± 2°C and 75% relative humidity (ICH Zone IV accelerated stability conditions), the immunobooster tablet formulation demonstrated excellent stability. There was no change observed in organoleptic characteristics, moisture content increased by only 0.13%, pH of 1% dispersion did not vary and DPPH scavenging reduced slightly from 78.56% to 77.80%. These results are supportive of a shelf-life of at least two years when

The carbon clearance assay provides a measure of phagocytic activity of the extract on in vitro incubation with murine peritoneal macrophages. Increase in phagocytic index compared to control is indicative of enhanced macrophage activation and acquired immunity. The following results were obtained:

What we've gathered so far fits together in a way that makes scientific sense, especially when thinking about how to build herbal medicines meant to support immunity. One after another, the six plant-based ingredients picked here bring something different - but clearly understood - to how the body's defenses respond; yet it's how they work together, guided by an old idea about synergy called *Rasayana*, that matters more than any single part. Together, they hit several biological pathways at once - not just one path followed alone - and that layered effect might be why using many herbs beats relying on just one substance, whether lab-made or from nature. Surprisingly, this broad reach stands out as the strongest benefit these mixed-plant formulas have compared to treatments built around solitary compounds.

What stood out was how the method kept heat-sensitive plant compounds intact during tablet formation. Instead of degrading them, the process held those elements steady while still building strong enough pills. Slowness in breaking apart when wet could have been an issue, especially with tannins gumming up absorption paths. Yet that delay never took hold because one ingredient - crospovidone - opened channels fast enough. Its amount made the difference, letting moisture slip past barriers others might create. Strength did not come at the cost of quick release, thanks to smart material pairing [2,9,13].

Looking at antioxidants needs careful thought. With 78.56% DPPH inhibition, this mix stands out compared to similar herbal blends in current studies. It matters because fighting oxidation ties closely to

DISCUSSION



immunity - when oxidants build up, they slow down lymphocytes, weaken natural killer cells, and create long-term irritation that dulls quick immune reactions. Removing harmful molecules helps each kind of immune cell work better, whether part of immediate or learned defenses [5,7].

One thing stands out - the phagocytic index jumped by 65%. That lines up with what earlier single-herb trials showed. It also backs the idea that combining herbs can work. Right at the start, macrophages react when exposed to something that wakes up immunity. When they fire up, it sets off a chain - cytokines get released, antigens are shown, T cells move in. What ties Ashwagandha's withanolides to Guduchi's tinosporin? Their actions fit together. Withanolides hit glucocorticoid receptors, easing stress-driven dampening of defenses. At the same time, tinosporin boosts how many pattern detectors sit on macrophage membranes [8,15,19].

One thing stands out: none of the studies looked at how the body reacts to these compounds inside living organisms, nor how they move through it - a common shortcoming when undergrads lead the work. Moving forward, solid proof from well-designed human trials will be needed before any real medical use in people with weak immune systems becomes possible. Starting each batch with plants means chemistry shifts naturally; consistency demands careful sourcing, strict checks on every supply run, so results stay reliable [14,25].

CONCLUSION

Now think about blending traditional herbs into modern medicine forms. Science backs this idea more every day. These plant-based tablets aim to support immunity using nature's blueprint. Take Indian gooseberry, known for fighting cell damage. It pairs with heart-leaved moonseed, which wakes up key defense cells. Together they work, yet each holds its own role. Holy basil joins in, lifting white blood cell response. Turmeric adds balance by calming excess inflammation. Its partner ginger strengthens resilience under strain. Each herb brings something different. Their actions overlap just enough to boost one another. Not everything blends smoothly at first glance. Still, their combined effect covers many angles of immune health. This mix doesn't rely on a

single pathway. Strength comes from variety. One plant handles stress adaptation. Another targets free radicals. They connect through biology, not chance. Results so far suggest real potential. The path forward stays rooted in evidence.

Nowhere else do herbal tablet tests show such steady results - these blend-based dissolvable pills pass every required check, from physical traits to chemical makeup. Because immune-boosting plant compounds often break down under heat, skipping high-temperature steps makes sense. Compression without melting keeps delicate substances whole, while cutting production complexity at the same time. Standards for purity, shelf life, germ control, and biological activity stay fully satisfied throughout.

Starting off, blending old Ayurvedic knowledge with modern drug research and real-world formulation techniques makes these multi-herb immunity products seem like sensible options for health support. Instead of standing still, they could fit into standard medical practice if proven properly. Moving forward, studies must focus on live biological testing, human trials involving vulnerable groups, alongside tracking how the body absorbs and processes these mixtures - this groundwork will shape approval paths by clarifying safe use, actual benefits, and correct amounts.

What happens next depends on how science responds. Public attention has shifted toward plant-based support for immunity, a change sharpened by what followed the pandemic. This shift brings weighty duty along with market potential. Formulations combining multiple herbs now face closer scrutiny. When studied thoroughly, they stand out more clearly. Recognition follows only when evidence is strong.

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