Development of Herbal Immunity Booster Formulation Using Standardized Extracts.

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ABSTRACT

The increased rates of infectious diseases, combined with growing consumer demand for natural remedies, have sparked interest in herbal immune-supportive formulations. This project developed and evaluated an immunity-enhancing herbal formulation based on the use of standardized extracts of medicinal plants that are widely recognized for their beneficial biological and immunological properties. The plants selected to create the formulation were Withania somnifera (Ashwagandha), Tinospora cordifolia (Guduchi), Ocimum sanctum (Tulsi), and Emblica officinalis (Amla). Standardized extracts from these herbs were used to create this polyherbal formulation in optimized quantities based on their known phytochemical composition and documented pharmacological activity. The standardized extracts were evaluated for quality attributes before mixing them into a polyherbal formulation using the optimized formulation. The finished formulation was evaluated based on physico-chemical properties, phytochemical content, free radical scavenging ability (anti-oxidant activity), microbial content, and stability under various storage conditions. The preliminary results of the phytochemical analyses indicated the presence of several constituents that produced immunomodulating and antioxidative effects, including alkaloids, flavonoids, phenols, tannins, glycosides, and saponins. Analyses of the antioxidant activity of the formulation demonstrated a high level of free radical scavenging activity, indicating that the formulation has the potential to reduce oxidative stress and promote immune health. The stability study of the formulation indicated that its physico-chemical properties and biological activity were consistent throughout the entire storage period. The standardized polyherbal formulation also demonstrated good organoleptic properties and complied with the standards of the pharmacopoeial quality system. The research shows that using standardized polyherbal extracts could be an excellent way to create safe effective and consistent formulations to enhance immunity These herbal products could function as adjuncts to supplementing with regard to immune response and general health. Continued research into these extracts in terms of pharmacology and clinical trials would help determine efficacy and safety for humans.

Keywords: Herbal formulation, Immunity booster, Standardized extracts, Polyherbal preparation, Antioxidant activity, Immunomodulatory activity, Medicinal plants.

INTRODUCTION

The immune system has a fundamental role in keeping people safe from harmful bacteria, viruses, parasites, and toxins, as well as environmental stressors. An optimal immune system ensures homeostasis in the body and lowers the risk of developing infectious or chronic disease. Over the last several years, increased public interest in preventive health care, combined with an awareness of the limitations of synthetic medicine, has led to significant growth in the use of herbal medicine and nutraceuticals to support immunity. Herbal products are of interest because they are “natural,” are generally considered safe, and can have multiple modes of action through different bioactive compounds. Herbal medicines have been used for hundreds (or thousands) of years for medicinal purposes worldwide, and in many traditional medical systems, such as Ayurveda, Traditional Chinese Medicine, and Unani, they have been applied as treatments to enhance or promote good health as well as to treat or cure disease. In fact, the World Health Organisation (WHO) estimates that 80% of people in the world rely on herbal medicines for their primary health care needs (WHO 2023). The therapeutic benefits of herbal

medicines are due to the presence of secondary metabolites such as alkaloids, flavonoids, terpenes, phenolics, tannins, and glycosides that possess antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties (WHO 2023) which are beneficial in stimulating your body's immune system and promoting general health and well-being.

With Ania *somnifera, Tinospora* cordifolia, Ocimum sanctum, and Emblica officinalis are some of the most well-known medicinal plants with significant immunomodulatory function. Withania somnifera contains withanolides, which have both adaptogenic and anti-inflammatory qualities and help regulate the immune response (Singh et al. 2011). Tinospora cordifolia contains many diterpenoid lactones and polysaccharides, which stimulate macrophage activation and increase antibody production (Sharma et al. 2012). Ocimum sanctum possesses eugenol and ursolic acid, which show both antioxidant and antimicrobial properties while enhancing host defense mechanisms (Pattanayak et al. 2010). Emblica officinalis provides a large amount of Vitamin C and polyphenols; therefore, it may contribute to improving both antioxidant status and immune competence (Baliga and Dsouza, 2011).

Polyherbal formulations rely on synergy; this means that combining multiple herbal extracts leads to greater healing than any constituent on its own. Polyherbal preparation have demonstrated advantages in effectiveness, lower toxicity, and increased/variety of pharmacological activities. Nevertheless, because of factors such as geographical region, growing conditions, and processing, the phytochemical compositions of herbal products can vary greatly, which have an impact on the quality and consistency of these products. Thus, standardized extracts must be utilised for quality control, to ensure batch-to-batch consistency, and to provide reproducible therapeutic outcomes from use of these products. Standardization involves identifying and measuring marker compounds and following established physicochemical and microbiological quality criteria (Mukherjee, 2019).

Chronic inflammation and oxidative stress are known to affect the immune system's ability to function properly and can also be involved in causing multiple diseases. Antioxidants from natural products such as medicinal plants can counteract reactive oxygen species (ROS) and therefore help protect immune cells from oxidative damage. Several published studies have shown that plant extracts containing major groups of phytonutrients (flavonoids and phenolic compounds) demonstrate very strong activities related to scavenging free radicals and enhancing the immune system (Kumar and Pandey, 2013). As such, the combination of standardized herbal extracts with recognized antioxidant abilities should lead to effective immunity-boosting formulations. Increased consumer preferences for natural products and preventive health care have contributed to the tremendous growth of the worldwide market for herbal supplements. Additionally, the demand for products using herbs to promote immunity has significantly increased due to the COVID-19 pandemic and the need for products with proven scientific standards. There has been an increase in the use of herbal medicine; however, such alternative therapies still require high-quality and scientifically-supported product options for efficacy, safety, and stability. As a result, this research project will develop a product based on the use of standardised extracts from different plant sources that contain active ingredients related to immunity, and characterise the product throughout its physical and chemical characteristics, active ingredient profile, ability to scavenge free radicals, bacteria killed – including identification of which strains were killed, and also by testing for the stability of the product over time/temperature. The goal is to provide clinical data to support a safe and effective herbal alternative to improving immune health and enhancing general wellness in patients.

Immune System and the Need for Natural Immunomodulators

There is a complex system of biological structures and functions, known collectively as the immune system, that protects the host from infection and maintains homeostasis. The immune system must function properly for an individual to avoid many traits associated with chronic disease, including infection. Recently, there has been an increased awareness of preventive medicine and a growing interest in non-pharmaceutical products that are believed to help modulate the immune response due to concerns over potential toxicities associated with synthetic pharmaceuticals. Accordingly, natural products as complementary health approaches have continued to gain popularity as they are considered generally safe, inexpensive, and abundant in the biologically active compounds found in these products that promote good health and function of the immune system (World Health Organization, 2023).

Role of Medicinal Plants in Immune Enhancement

People have relied on medicinal plants for hundreds of years through different forms of natural medicine such as Ayurveda, Unani and Traditional Chinese Medicine. Medicinally, each of these groups contains different types of phyto-chemicals with many different types and combinations of similar chemicals including alkaloids; flavonoids; tannins; terpenoids; glycosides; and phenolic compounds, but all share a common purpose of providing benefit physiologically through their antioxidant, anti-inflammatory, anti-microbial, and immunomodulatory properties. Because of this benefit to our immune systems and providing us with additional resistance against various illnesses, Ekor (2014) states that the use of herbal medicines as a primary source of health care continues around the globe due to the positive effects they have on treating conditions as well as relatively low rates of side effects.

Therapeutic Importance of Selected Herbal Extracts

A number of different medicinal plants have been evaluated by scientists and have been shown to be beneficial in providing enhanced immune function (e.g., Asad et al., 2019). Some have even been shown to possess both adaptogenic effects and immune-modulating effects (e.g., Asad et al., 2020). One such plant, Withania somnifera (*or "Ashwagandha*"), is known to contain certain compounds—specifically, withanolides—that have been found to enhance cellular immunity and to counteract immune-suppressing stressors (Singh et al, 2011). Additionally, Tinospora cordifolia (or "Guduchi") has also been shown to contain compounds that stimulate antibodies and macrophage function, thereby enhancing an individual's immune response (Sharma et al, 2012). In a similar manner, Ocimum sanctum (or "Tulsi") contains compounds—specifically, eugenol and other phenolic compounds—that have been found to possess antioxidant and antimicrobial properties, thus providing potential protective effects against oxidative injuries and infections (Pattanayak et al, 2010). Finally, Emblica officinalis (or "Amla") has been shown to be high in Vitamin C, tannins, and polyphenols and has been found to improve antioxidant status and overall immune competence (Baliga & D'Souza, 2011). Therefore, these herbs have been postulated to have synergistic therapeutic benefits and to provide more extensive pharmacological activity when used in combination.

Importance of Standardized Extracts in Herbal Formulations

The variance of phytochemicals in herbal medicines caused by environmental, harvest, or manufacturing processes is one of the main difficulties regarding their use, as this affects the quality/productivity of herbal medicines. Therefore, it is necessary to standardize these herbal extracts to ensure that they are produced with similar levels of effectiveness, safety, and reproducibility. This is achieved by producing herbal extracts that have known amounts of active chemistry and applying strict quality control measures to achieve uniformity between batches for each herb's compositions. Mukherjee (2019) notes that the standardization of herbal extracts is a very important part of developing high-quality herbal medicines and ensuring the quality assurance of products, which are scientifically acceptable and reliable.

Antioxidant Activity and Immunological Benefits

Excessive production of reactive oxygen species can result in oxidative stress, which negatively influences our immune system and can contribute to a variety of diseases. Therefore, many medicinal plants provide natural antioxidants which help neutralise free radicals and provide protection for our immune system from the effects of oxidative damage. Many of these herbal extracts contain polyphenols, flavonoids and vitamin C and therefore exhibit strong antioxidant properties, thereby providing support to normal immune function. Phytochemicals with antioxidant properties have a very important role to enhance immunity and alleviate inflammation are included in herbal formulations (Kumar and Pandey, 2013). Thus, the addition of standardised extracts high in antioxidants into herbal formulations may represent an excellent opportunity for developing effective immunity-enhancing agents.

Growing Demand for Herbal Immunity Boosters

There has been a significant rise in the demand for herbal supplements and nutraceuticals over the last ten years

due to the increasing preference for natural health care solutions. The COVID-19 Pandemic has also brought to the forefront the need to maintain immune health and has accelerated the use of herbal immune boosters by consumers around the world. As a result of this emerging consumer trend, researchers and pharmaceutical developers are putting more emphasis on producing standardized polyherbal formulations with greater efficacy and safety. This desire for high-quality, scientifically validated herbal products that offer preventive health benefits has helped drive growth and innovation within the overall nutraceutical industry.

Rationale and Scope of the Present Study

In light of the medicinal values of plants and ever-increasing consumer needs for herbal immunity boosters, this project work aims to develop a herbal immunity enhancer formulation from standardized extract of Ashwagandha, Guduchi, Tulsi and Amla which exert complementary action and it contains quality, safety and reproducibility. Formulation would be evaluated for its physicochemical characteristics, phytochemical profiling, antioxidant activity, microbial profile and stability, to establish it as one of the promising and effectual adjunct health Supplement for improvement of health and enhancing immune status.

MATERIALS AND METHODS

Study Design

A research experimental laboratory approach has been performed to develop and screen a polyherbal immune booster formulation comprising of standard extracts of specific medicinally important plants. Herbal extracts were procured, extracted, and standardized, formulated, subjected to various physiochemical characterization studies, phytochemical tests, antioxidant evaluations, and antimicrobial and stability studies.

Materials

With Ania somnifera (*Ashwagandha*), Tinospora cordifolia (*Guduchi*), Ocimum sanctum (*Tulsi*), and Emblica officinalis (*Amla*) standardized extracts were purchased from authorized suppliers and authenticated on the basis of pharmacopoeial standards. Analytical grade chemical and reagent such asmethanol, ethanol, distilled water, ferric chloride, Mayer's reagent, Drage Dorff's reagent, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were procured from a noted chemical supplier. Chemicals were purchased of an analytical grade.

Preparation of Polyherbal Formulation

Standardized extracts (30% ashwagandha, 25% guduchi, 20% tulsi, 25% amla) weighed accurately and formulated as according to their optimized immunomodulatory properties and standardized doses. The extracts are processed together so as to form fine homogenized powder. The resultant mixture is processed by adding excipients like microcrystalline cellulose and magnesium stearate for ensuring better flowability and stability of formulation. The formulated product is maintained in an air tight packed condition in absence of light and moisture.

Physicochemical Evaluation

The resulting formulation was analyzed for some of the physicochemical parameters: Organoleptic properties, pH value, loss on drying (moisture content), bulk density, tapped density, angle of repose, Carr's index and Hausner ratio of formulated granules. All these studies were carried out using appropriate Pharmacopoeial methods as per guidelines for determination of flow parameters (moisture content by loss on drying methods).

Preliminary Phytochemical Screening

Phytochemical qualitative test conducted to detected compounds were performed based on standard protocols for detection of phytochemicals alkaloids, flavonoids, tannins, phenols, saponins, steroids, triterpenoids, saponin, glycoside. The phytochemical constituent presence was tested and indicated by formation of color

precipitate based on specific chemical tests such as test by the reagents from Mayer's and Dragendorff's reagents (alkaloid), test from ferric chloride (tannins and phenols) and foam test (saponin) as well as shinoda's test (flavonoids).

Determination of Total Phenolic and Flavonoid Content

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method and expressed as milligrams of gallic acid equivalents per gram of extract. Total flavonoid content was determined using the aluminum chloride colorimetric assay and expressed as quercetin equivalents. Absorbance values were measured using a UV-visible spectrophotometer, and the concentration of phenolic and flavonoid compounds was calculated from standard calibration curves.

Antioxidant Activity Assay

The antioxidant potential of the polyherbal formulation was evaluated using the DPPH free radical scavenging assay. Different concentrations of the formulation extract were mixed with DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid served as the reference standard. The percentage inhibition of DPPH radicals was calculated using the equation:

$$\text{Percentage inhibition (\%)} = [(Ac - As)/Ac] \times 100$$

where Ac represents the absorbance of the control and As represents the absorbance of the sample.

Microbiological Evaluation

Microbiological quality testing was done according to standard methods of the WHO and official pharmacopoeias. The Total Aerobic Microbial Count and the Total fungal count were enumerated by the standard plate method using the nutrient and the Sabouraud's dextrose agar media. Pathogenic microorganisms *viz E. Coli, Salmonella spp*, Staphylococcus aureus and Pseudomonas aeruginosa were monitored on the selective media and were then counted and compared with permissible limits.

Stability Studies

In order to evaluate the physical and chemical stability of the developed formulation, accelerated stability studies were performed. The sample was stored in closed containers at 40 ± 2°C and 75 ± 5% relative humidity for three months following ICH guidelines. The color, odor, moisture content, pH and antioxidant activity of the sample were measured periodically and the results obtained were recorded to identify the shelf-life and storage conditions of the final product.

Statistical Analysis

All experiments were repeated three times in triplicates and data were expressed as the mean SD. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 26.0. Comparisons between groups were analyzed with one-way analysis of variance (ANOVA) and were considered statistically significant for $p<0.05$.

RESULTS AND DISCUSSION

The goal of the current study was the creation and testing of an herbal immune booster supplement that meets

both modern standards for quality control and contains a mix of herbs traditionally used in herbal medicine. A final product was successfully created with varying amounts of ashwagandha, *guduchi, tulsi*, and amla extracts and has many naturally occurring chemical compounds that are known to stimulate the immune system and protect against free radicals. In terms of the physical characteristics of the product, the final formulation yielded a very fine ground brown powder with a distinct odor, had a pH of 6.7 ± 0.1, had moisture content of 4.2 ± 0.3%,

contained acceptable flow characteristics, and can be further processed into capsules and/or tablets due to the excellent flowability of the powders as indicated by the Carr's index (14.8 ± 0.5%), Hausner ratio (1.17 ± 0.02), and angle of repose (28.4 ± 1.2°). Several important secondary metabolites were demonstrated by preliminary phytochemical analysis. The formulation was tested and resulted in positive alkaloid, flavonoid, phenolic, tannin, glycoside, saponin, steroid, and terpenoid detection.

The presence of bioactive compounds suggests the formulation has multiple therapeutic functions, such as antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory. The results from physicochemical, phytochemical, antioxidant, microbiological, and stability studies revealed that the developed herbal immunity boosting formulation has desirable quality characteristics with an excellent antioxidant ability and adequate stability. These results support the probable use of the herbal supplement as a natural supplement to improve immunity and improve the overall health.

Table 1. Composition of Polyherbal Immunity Booster Formulation

Ingredients	Botanical Name	Percentage (%)	Major Active Constituents
Ashwagandha	Withania somnifera	30	Withanolides
Guduchi	Tinospora cordifolia	25	Diterpenoid lactones
Tulsi	Ocimum sanctum	20	Eugenol, flavonoids
Amla	Emblica officinalis	25	Vitamin C, polyphenols

The formulation was created using four herbal extracts standardized to the same level and proportioned correctly. The largest portion of the formulation was ashwagandha (30%) because of its proven adaptogenic and immunomodulatory properties. The next two largest portions were Guduchi and Amla, each contributing 25%. Tulsi accounted for the remaining 20%. This combination resulted in the presence of multiple types of bioactive compounds with proven synergistic antioxidant properties and immune enhancement properties (withanolides, flavonoids, polyphenols, and diterpenoid lactones).

Table 2. Physicochemical Characteristics of the Developed Formulation

Parameter	Observed Value
Appearance	Fine brown powder
Odor	Characteristic aromatic
pH (1% solution)	6.7 ± 0.1
Moisture content (%)	4.2 ± 0.3
Bulk density (g/cm³)	0.46 ± 0.02
Tapped density (g/cm³)	0.54 ± 0.03
Carr's Index (%)	14.8 ± 0.5
Hausner Ratio	1.17 ± 0.02
Angle of Repose (°)	28.4 ± 1.2

The physicochemical testing indicated that the product had acceptable characteristics with a typical aroma and colour that was brown in colour. The moisture content was < 5% indicating good stability when stored. The flow rates were acceptable and were verified by Carr's index (14.8%), Hausner ratio (1.17) and the angle of repose (28.4°) showing excellent powder flow properties for the manufacture of either capsules or tablets.

Table 3. Preliminary Phytochemical Screening

Phytochemical Constituents	Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Phenolic compounds	Present (+)
Tannins	Present (+)
Glycosides	Present (+)

Saponins	Present (+)
Steroids	Present (+)
Terpenoids	Present (+)

Several biologically active compounds were found to be present in the developed formulation based on preliminary phytochemical analyses. The high levels of flavonoids & phenolics are known for their high levels of antioxidant potential. Alkaloids, tannins and flavonoids, which are also included in the formulation support the therapeutic benefits expected from this product. These phytoconstituents exhibit antimicrobial (ability to kill or inhibit bacteria), anti-inflammatory (reduce swelling) and immunomodulatory (support your body's own defense mechanisms) activity together.

Table 4. Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity

Parameter	Value
Total phenolic content	86.4 ± 2.5 mg GAE/g
Total flavonoid content	53.7 ± 1.8 mg QE/g
DPPH Radical Scavenging Activity (%)	82.6 ± 2.1
IC50 value (µg/mL)	41.2 ± 1.4
Standard Ascorbic Acid Inhibition (%)	90.4 ± 1.5

The produced formulation displayed a substantial antioxidant activity; the DPPH radical scavenging activity was 82.6%. The total phenolic and flavonoid contents were 86.4 mg GAE/g and 53.7 mg QE/g, respectively, indicating that there is a high concentration of antioxidant phytochemicals present. The IC50 value of 41.2 µg/mL demonstrated that it has strong free radical scavenging ability. While slightly lower than that of the standard ascorbic acid, thexfomulated product exhibited a great deal of antioxidant potential, which has the potential to support maintaining a healthy immune system, as well as help to reduce oxidative stress.

Table 5. Microbiological Evaluation and Stability Study Results

Parameter	Initial	After 3 Months
Total aerobic microbial count (CFU/g)	1.8×10^2	2.1×10^2
Total fungal count (CFU/g)	0.7×10^2	0.9×10^2
Escherichia coli	Absent	Absent
Salmonella spp.	Absent	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Color	No change	No change
Odor	No change	No change
Antioxidant activity (%)	82.6 ± 2.1	80.8 ± 2.3

Assessment of the microbiological quality of the developed formulation demonstrated that it met the accepted limits for microorganisms and did not contain any pathogenic organisms, including Escherichia coli, Salmonella spp., Staphylococcus aureus, and Pseudomonas aeruginosa. The results of the accelerated stability studies conducted over a period of three months showed that the formulation did not undergo any significant physical changes in terms of colour, odour or antioxidant activity. The small decrease in antioxidant activity from 82.6% to 80.8% indicates that it will maintain good chemical stability. Therefore, the results suggest that the polyherbal formulation is of acceptable microbiological quality and will remain stable in storage, thus providing an adequate basis for its potential use as a safe, effective herbal immunity booster.

DISCUSSION

This study provided evidence of the successful formulation of a polyherbal immunity booster using standardized extracts of four different herbs: Ashwagandha (*Withania somnifera*), Guduchi (*Tinospora cordifolia*), Tulsi (Ocimum sanctum), and Amla (*Emblica officinalis*). The herbal extracts were selected as they have historically

been used in traditional herbal medicine systems because of their immunomodulatory, antioxidant, and adaptogenic actions; furthermore, many of these actions have been validated by modern scientific studies. Since the extracts were carried out using the same approach (i.e., standardized methods), the resulting herbal products had a uniform composition of phytochemicals; therefore, greater reproducibility and higher-quality products were obtained with the same type of phytochemical profiles than those that may not have been prepared in such an organized and scientifically valid manner. Also, Mukherjee (2019) reported similar results and concluded that standardisation is an integral part of producing quality herbal medicine products through the measurement and validation of quality control parameters during the product development process.

The results from the physicochemical assessments indicate that the physical characteristics of the product demonstrate excellent flowability and organoleptic properties. In addition, the low moisture content and the accepted values for bulk density, tapped density, Carr's index, Hausner ratio, and angle of repose were indicative of the combined characteristics of the formulation providing an overall stable product that is easily handled during processing into final solid dosage forms (e.g., tablets/capsules). Previous studies have illustrated that maintaining acceptable physicochemical characteristics is crucial to maintaining the long-term stability of herbal products (Kokate et al., 2010).

The results of the initial phytochemical screening showed that the formulation contains several phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids, and terpenes. There are well-documented studies discussing the biological effects of these secondary metabolites. Flavonoids and phenolic compounds are known to neutralize free radicals (reactive oxygen species) and enhance host defense capabilities (Kumar and Pandey, 2013). Thus, the aforementioned phytochemical compounds in this formulation are likely to synergistically contribute toward enhancing immune function and overall health.

The results of the quantitative analyses indicated that the formulation contains significant amounts of antioxidant compounds, with the total phenolic and flavonoids content being determined. Results from the DPPH radical scavenging assay demonstrated that the formulation exhibits significant levels of antioxidant activity, indicating that it has the capacity to scavenge free radicals and, therefore, protect cells from oxidation. Oxidative stress has been shown to result in immunological dysfunction and to play a role in the development of various chronic diseases. Therefore, the antioxidant properties identified in this research could be utilized to provide support for the immune response and for reducing inflammation. The antioxidant and protective effects of the plant Emblica officinalis, demonstrated by Baliga and D'Souza (2011), and the antioxidant activity of Ocimum sanctum, documented by Pattanayak et al. (2010), support these findings.

Adaptogenic and immunostimulatory properties have long been attributed to Ashwagandha. When Withania somnifera produces its withanolide compounds, they help support cellular immunity, stimulate macrophages, and alter the levels of cytokines (Singh et al., 2011). Similarly, polysaccharides and diterpenoid lactones found in Tinospora cordifolia can help with phagocytic activity and enhance immune response. Guduchi may also enhance humoral and cell-mediated immunity by increasing infection resistance (Sharma et al., 2012). The combination of these two herbs is likely to act together, thus maximising therapeutic value.

Microbiological evaluation of the formulation has shown that the formulation met permissible microbiological standards and was free of any pathogenic organisms such as Escherichia coli, Salmonella spp., Staphylococcus aureus, and Pseudomonas aeruginosa. Therefore, the results provide evidence of the microbiological safety of the formulation and that proper routine manufacturing practices and quality assurance have been followed. The World Health Organisation recommends that similar microbiological standards be adhered to for herbal medicines to ensure product safety and efficacy (WHO, 2023).

Accelerated stability investigation revealed that the formulation remained stable in terms of physical attributes and has maintained antioxidant levels throughout storage, with only a small decrease observed in free radical scavenging capacity after three months. Stability is indicated by no significant alterations in colour, smell, or microbial load, meaning that this formulation is stable and thus will have an approved shelf life. Stability studies play an important role in the development of herbal products, as phytoconstituents may degrade under various environmental conditions. The current research findings indicate that this formulation will be acceptable for long-term storage and for further commercialisation.

This study supports the hypotheses that the polyherbal formulation being developed has favorable physicochemical characteristics, extensive number of phytochemicals, strong antioxidant properties, microbiological safety and adequate stability. The therapeutic benefits of the polyherbal formulation are further enhanced through the presence of synergistic benefit obtained from each of the selected medicinal plants used to create the formulation, and therefore supports its potential as a natural immune enhancing dietary supplement. The current study focused solely on laboratory assessment; additional studies are needed to evaluate the in vivo effect, toxicity evaluation or randomised clinical trials, to prove its effectiveness or safety in human subjects. Further research will provide additional evidence in favour of the application of standardized herbal formulations for preventive healthcare or immune enhancements.

CONCLUSION

A formulation of a polyherbal immunity booster was developed within this study using standardized formulations of the following herbal extracts: *Withania somnifera* (Ashwagandha), Tinospora cordifolia (*Guduchi*), Ocimum sanctum (*Tulsi*) and Emblica officinalis (*Amla*). Standardization of the extracts also provided consistency in the phytochemical composition of the finished formulation, thus enhancing the overall quality and reproducibility of the end product. Evaluation of physicochemical properties found that the above-mentioned formulation had appropriate organoleptic characteristics and sufficient flow characteristics for continued pharmaceutical development. In addition, the preliminary phytochemical analyses confirmed the presence of many bioactive compounds (alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids and terpenes) in each of the herbal extracts that possess antioxidant, anti-inflammatory, antimicrobial and immunomodulatory abilities. Through quantitative analyses, moderate quantities of phenolic & flavonoids compounds were determined to be present in the formulation; moreover, the DPPH assay showed significant scavenging activity towards free radicals suggesting a high antioxidant capacity and ability to promote immune system functionality. Microbial testing showed that the formulation had acceptable standards of quality, was free of any pathogenic microbes, and was therefore safe to use. There were few changes in the physical and chemical properties and antioxidant activity after accelerated stability testing. These results indicate that the formulation has satisfactory stability for shelf life and will retain its biological activity over time. The results of this research indicate that the polyherbal formulation developed has excellent physicochemical characteristics, a high level of phytochemical compounds, strong antioxidant activities, is microbiologically safe to use, and a good degree of stability. These features support the use of this formulation as a natural supplement to promote immunity, which could augment the use of preventive healthcare. Additionally, the combined effects of Ashwagandha, Guduchi, Tulsi, and Amla create an excellent alternative to synthetic immune boosters, emphasising the need for using standardised herbal products in contemporary treatment practices.

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