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Combination Effects of Herbal Formulations for Enhanced Immune Support: A Comprehensive Review

Shriyesh Kumar Pandey[1], Neha[2]

[1]Department of Pharmacy, Six Sigma Institute of Technology and Science, Jafarpur, Uttarakhand

[2]Assistant Professor, Department of Pharmacy, Six Sigma Institute of Technology and Science, Jafarpur, Uttarakhand

Abstract:
Herbal formulations for boosting immune support are becoming more popular as a result of growing interest in natural therapies. This thorough analysis examines the combined effects of several herbal preparations, emphasizing their synergistic qualities, modes of action, and general benefits on immunological health. A wide range of bioactive substances that cooperate to regulate immune responses, lower inflammation, and fight oxidative stress are frequently found in herbal preparations. Important herbs that have important functions in boosting immune function through antioxidant activity, cytokine regulation, and antiviral actions include clove (Syzygium aromaticum), ginger (Zingiber officinale), garlic (Allium sativum)the significance of comprehending how various herbs interact with one another since these mixtures can increase therapeutic efficacy beyond that of their individual parts

Keywords: Herbal Formulations, Immune Support, Antioxidant Activity, Quality Control

Corresponding Author: Shriyesh Kumar Pandey†, Department of Pharmacy, Six Sigma Institute of Technology and Science, Jafarpur, Uttarakhand

Introduction

In many civilizations around the world, herbal medicine items have been used as the main source of healing for generations. (1) These goods, which are made from plants and plant-derived materials, provide a wealth of bioactive chemicals that may have medicinal uses.(2) Herbal remedies are used in a variety of traditional systems, including indigenous healing methods, Ayurveda, and traditional Chinese medicine (TCM).(3) The presumed natural origin and historical use of herbal medicinal items are major factors in their continued appeal. Herbal treatments have long been used in many countries to treat a variety of illnesses and enhance general wellbeing.(4) This traditional knowledge has been passed down through generations, offering valuable insights into the healing properties of various plant species. (5) Products made from herbal medicines have shown promise in treating a range of illnesses, such as respiratory diseases, chronic pain, digestive issues, and immune system support.(6) Their bioactive constituents, such as alkaloids, flavonoids, terpenes, and polyphenols, can interact with biological systems, offering potential therapeutic benefits.(7) However, the growing demand for herbal medication products also raises concerns about quality control, safety, and efficacy (8). Ensuring the consistent quality and standardization of herbal products is crucial to guarantee their safety, efficacy, and reproducibility.(9) Rigorous quality control measures are essential to protect the health and safety of consumers.(10) By implementing stringent testing and quality assurance protocols, potential risks such as contamination, adulteration, and the presence of harmful substances can be minimized, ensuring that the herbal medication products are safe for consumption.(11) Insufficient quality control can pose serious safety risks to consumers. Contaminants, adulterants, or incorrect formulations in herbal medication products may lead to adverse reactions, toxicity, or other health complications.(12) Inadequate quality control may result in inconsistent levels of active compounds in herbal medication products.(13) This inconsistency can lead to variable therapeutic effects, making it challenging for healthcare professionals to prescribe and manage patient treatments effectively.(14) Quality control measures help maintain consistency in the composition and potency of herbal medication products.(15) Through standardized manufacturing processes and testing methods, the levels of active compounds can be monitored and controlled, ensuring that the products deliver the desired therapeutic effects consistently.(16) This commitment helps build trust among consumers, healthcare professionals, and regulatory authorities. The purpose of the review is to provide. comprehensive overview of the importance, methodsconsiderations involved in ensuring the quality, efficiency, and safety of herbal medication product.

Mechanisms of Action

Datura stramonium Linn exhibited significant anti-inflammatory and antimicrobial activity due to rich and diverse presence of flavonoid and phenolic content.(17) It was traditionally used in smooth painful wounds and sores. The plant was also used as a poultice in treating fistulas, abscesses wounds and severe neuralgia.(18) The plant bioactives of *Plumbago zeylanica* exhibited anti-bacterial, anti-malarial and anti-fungal activities(19) and has been found to have efficacy, traditionally in reducing inflammation.(20) *Argemone mexicana* Linn has its own significance in traditional medicine system. It have been used for decades for the treatment of various ailments like dermatological diseases, warts, inflammatory complications and microbial infections. Alkaloids are most abundantly found in these species and contribute in the management of diverse categories of ailments.(21)

1. Activation of Immune Cells: Naive T cells are activated upon interaction of their surface TCRs with pathogen fragments in complex with major histocompatibility complex (MHC) molecules on the surface of target cells. Activation is accompanied with formation of the immunological synapse [22], coalescence of lipid rafts in the T cell membrane [23] and activation of Lck and Fyn [24], [25], [26], which phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3 subunits of the TCR complex.ζ-chain-associated protein of 70 kDa (Zap-70) is then recruited to the phosphorylated ITAMs and thereby brought into the proximity of Lck, which activates it byphosphorylation of Y493 [27–29]. Subsequently, Zap-70 phosphorylates linker for activation of T cells (LAT) [30,31]. LAT functions as a docking site for a number of adaptor and signaling molecules and is essential for downstream TCR signaling [31–34]. The signaling pathways culminate in the activation of multiple transcription factors of the nuclear factor κB (NFκB)/Rel/nuclear factor of activated T cells (NFAT), activator protein 1 (AP-1) and cAMP response element (CRE)-binding protein (CREB)/ activation transcription factor (ATF) families [35], and promote transcription of a number of genes important for immune activation, e.g. IL-2 [36]. A costimulatory signal is required to induce IL-2 synthesis in naive T cells, mediated through binding of the T cell surface protein CD28 to B7.1/B7.2 expressed on target cells. Absence of appropriate CD28 coengagement results in T cell anergy [37]. for an illustrative overview of T cell activation. IL-2 is secreted and binds to IL-2 receptors on the T cell surface in an autocrine manner to drive clonal expansion and acquisition of effector or memory function. CD8+ T cells differentiate into cytotoxic effector T cells, while CD4+ T cells differentiate into Th1 cells, which produce IFNγ and promote macrophages and cell-mediated immunity, or Th2 cells, which secrete IL-4, IL-5 and IL-13 and promote B cells and humoral immunity, depending on the cytokine environment [38]. Migration of Th2 cells to the lung is key to the manifestation of allergic asthma. Allergen-sensitized mice that lack the β-arrestin gene do not accumulate Th2 cells in their airways [39]. More recently, additional Th cell subsets that play roles in immune regulation have emerged, including regulatory T (TR) cells [40].

Cytokine Modulation

Cytokines, a large group of soluble extracellular proteins or glycoproteins, are key intercellular regulators and mobilizers. Classified into family groups (e.g., interleukins, interferons, and chemokines) based on the structural homologies of their receptors, these proteins were initially believed to act primarily as antiviral.(40) They are now seen to be crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis, and developmental as well as repair processes.(41) Their secretion, by virtually every nucleated cell type, is usually an inducible response to injurious stimuli.(41) In addition, cytokines provide a link between organ systems, providing molecular cues for maintaining physiological stability.(42)

Medical literature of the last several decades reveals an array of conditions, from cardiovascular disease to frailty, whose onset and course may be influenced by cytokines The understanding of stimuli that invoke cytokine secretion has expanded. Besides chronic infections, negative emotions and stressful experiences have been shown to stimulate production of proinflammatory cytokines.(43)

The diverse and far-reaching influences of these proteins can be seen in the central nervous system (CNS); cytokines cause the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral shifts.(44) 6 Abnormal cytokine production has been demonstrated in neuropsychiatric disorders such as attention deficit hyperactivity disorder, obsessive-compulsive disorder, and anorexia nervosa.Cytokines also appear to play a role in depression, schizophrenia, and Alzheimer’s disease.(45)

As models of physiology continue to develop beyond compartmentalized organ systems, elucidation of the global activity of cytokines offers further support to an expanding understanding of cell-to-cell communication. The inflammatory processes of cardiovascular disease are one such example. Beyond leukocytes, the liver, heart, vessel walls, and adipose tissue are known to produce cytokines; thus any of these tissues may potentially contribute to the inflammatory nature of cardiovascular disease.(46)

Therapeutic application of cytokines in clinical medicine has rapidly surpassed the FDA’s 1986 approval of an interferon (IFN) agonist for the treatment of hairy cell leukemia. In 2001, an antagonist to tumor necrosis factor (TNF), a pivotal cytokine in the pathogenesis of rheumatoid arthritis (RA), was described as one of the most important advances in RA treatment. (47)

Antioxidant Activity

Antioxidant factors found in plants are based upon constituent nutrients with demonstrated radical-scavenging capacities as well as upon non-vitamin or mineral substances. So, in addition to alpha-tocopherol, ascorbate, carotenoids, and zinc, plantbased medicines may contain flavonoids, polyphenols, and flavoproteins. Further, some plants or specific combinations of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by increasing superoxide dismutase activity in various tissue sites.(48)

These groups of compounds are substances that may exert cell-protective action by more than one biochemical mechanism. In addition to antioxidant properties per se, cancer-protective factors are found in many plants including some fruits, vegetables, and commonly used spices and herbs. They can be divided into several different groups based on their chemical structure, e.g., polyphenols, thiols, carotenoids and retinoids, carbohydrates, trace metals, terpenes, tocopherols and degradation products of glucosinolates (isothiocyanates, indoles and dithiothiols) and others. These groups of compounds are substances which may exert their cancer-protective action by more than one biochemical mechanism. The biochemical processes of carcinogenesis are still not known in detail and probably vary with the cancer disease in question. Accordingly, the description of the biochemical backgrounds for the actions of cancer-protective factors must be based on a simplified model of the process of carcinogenesis. The model used in this presentation is a generalized initiation-promotionconversion model, in which initiators are thought to be directly or indirectly genotoxic, promoters are visualized as substances capable of inferring a growth advantage on initiated cells and converters are believed to be genotoxic, e.g. mutagens, clastogens, recombinogens etc. Experimental evidence for the mechanisms of action of cancer-protective agents in fruits and vegetables that protect against initiation include the scavenging effects of polyphenols on activated mutagens and carcinogens, the quenching of singlet oxygen and radicals by carotenoids, the antioxidant effects of many compounds including ascorbic acid and polyphenols, the inhibition of activating enzymes by some flavonols and tannins, the induction of oxidation and of conjugation (protective) enzymes by indoles, isothiocyanates and dithiothiones, the shielding of sensitive structures by some polyphenols and the stimulation of DNA-repair exerted by sulphur-containing compounds. Mechanisms at the biochemical level in antipromotion include the antioxidant effects of carotenoids and the membrane stabilizing effects reported with polyphenols, the inhibition of proteases caused by compounds from soybeans, the stimulation of immune responses seen with carotenoids and ascorbic acid, and the inhibition of ornithine decarboxylase by polyphenols and carotenoids. A few inhibitors of conversion have been identified experimentally, and it can be argued on a theoretical basis, that many inhibitors of initiation should also be efficient against conversion. The mechanisms of anticarcinogenic substances in fruits and vegetables are discussed in the light of cancer prevention and inhibition [48]

Plant antioxidants are more than mere supporting players in the battle against cellular damage and disease. As folklore has long instructed, certain plants play specific roles in disease prevention and treatment. A well known hepatic antioxidant, silymarin, from the milk thistle (Silybum marianum), for example, inhibits liver damage by scavenging free radicals among other mechanisms.(49)

Key Herbal Formulations

1. Clove:

Clove (Syzygium aromaticum), from the Myrtaceae family, is one of the most effective antimicrobial and antioxidant herbs. This herb is one of the traditional herbs primarily local to Asia and Africa. Based on the bioactive components of clove such as eugenol, eugenyl acetate, α-humulene, 2-heptanone, and β-caryophyllene, it can display many pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, antimutagenic, anticancer, and anti-allergic properties. These bioactive components allow clove to demonstrate one of the highest potent antioxidant activities among other herbal medicines. Previous studies have reported the sufficient antibacterial property of clove extract and oil against different strains of bacteria (Gram-positive and Gram-negative) [50,51]that clove has antimicrobial activity against many bacteria including Listeria monocytogenes, Klebsiella pneumoniae, S. aureus, E. coli and S. Typhimurium [52,54]

Clove based on eugenol plays an antimicrobial role. The antimicrobial mechanism of action of eugenol is that, at first, the eugenol molecule with high solubility can participate in the cytoplasmic membrane. Then, it creates disturbance as a consequence of its OH group. Finally, it can pass through the hydrophilic proportion of the cell. After that, the OH group of eugenol can bind to proteins of the membrane of bacteria and can permeate the fundamental cell components [55,56] . Clove extract with various amounts (1 and 3 $mg\ mL^{-1}$) have been demonstrated to experience a great antimicrobial impact on S. typhi and E. Coli [57]. Clove has strong antioxidant effects that can naturalize ROS and other free radicals in lipid chains. Therefore, they inhibit further oxidation of lipids [58]. Researchers have also observed that clove’s extract could inhibit the malondialdehyde formation from horse blood plasma oxidation [59]. Other researchers have reported the maximum antioxidant activity of clove against DPPH (2, 2-diphenyl-1-picryl hydracyl), than BHA (butylated hydroxyanisole), and BHT (butylated hydroxytoluene) radicals [60]. Similarly, the result of one research has shown the antioxidant activity of eugenol of clove extract against DPPH, ABTS and superoxide radicals [61]. Other studies have reported strong antioxidant activity of this herb against DPPH when compared to vitamin C [62]. The antiviral activity of this plant has been reported against different viruses such as herpes adenovirus, poliovirus, and coxsackievirus [63].

2. Ginger:

Ginger (underground rhizome of Zingiber officinale roscoe and herbaceous perennial plants) is one of the essential herbal medicinal plants from the Zingiberaceae family [64]. Ginger (the rhizome of Zingiber officinale) is native to Asia and has been used as a medicine for more than two thousand years around the world [65–68]. Ginger contains polyphenol components, including phenolic acids, gingerols, paradols, and shogaols. These principal components are responsible for its biological properties such as antioxidant, antidiabetic, antimicrobial, renoprotective, antihypertensive, antiulcer, anti-inflammatory, cardiovascular, analgesic, and gastrointestinal activities [69,70,71–73]. The antioxidant activity of ginger is related to the chemical compounds present in ginger such as zingiberene, zingerone, shogaols, and gingerols [74]. Some studies have analyzed the antioxidant activities of ginger and its components in numerous in vivo and in vitro lab experiments. Some researchers have demonstrated the potential antioxidant properties of ginger extract [75–77]. One study on rats has shown that ginger extract have antioxidant effect [78]. This herbal medicine also affects insulin secretion, insulin action, or both [79–81]. It can affect type one and type two diabetes because it can inhibit the metabolism of carbohydrates and lipids [82–84]. Extract of ginger shows high antimicrobial activity against dissimilar strains of bacteria such as S. aureus, E. coli, and Salmonella typhi [85,86]. Ginger has been used in several countries as a one of the most significant anticancer medicines based on the exogenous antioxidant activity of this plant. Therefore, it can be used as a treatment of diseases caused by free radicals [87]. Furthermore, the safety of using ginger as an antibiotic medicine has been investigated. In general, ginger has been known as a safe herbal medicine with pharmacological activity [88].

3. Garlic:

Garlic (Allium sativum) is an herbal medicine belonging to the Amaryllidaceae family [89]. Garlic is native to Central Asia, especially Iran [90]. Garlic has been demonstrated to possess biological activities including antioxidant, immunomodulatory activities, antidiabetic, anticancer, antibacterial, cardioprotective, and anti-inflammatory effects. The major components of garlic are phenolic, polysaccharides, and organosulfur contents. It also contains saponins, amino acid, flavonoids, vitamins A and C, B-complex vitamins, and minerals [91,92]. These chemical components are the reason for the biological activity of garlic. Garlic has been known as a natural antioxidant and can inhibit the harmful effects of free radicals in cells [93–95]. Antioxidant materials are naturally found in different plants and can neutralize free radicals through electron donation and by converting these harmful molecules to harmless products. Garlic is one of the traditional medicines with antimicrobial and antioxidant characteristics [96]. Numerous researchers have demonstrated the antioxidant property of garlic which is due to the presence of some chemical components including organosulfur, and phenolic compounds [97]. Garlic has a high content of phenolic compounds which are the reason for the high antioxidant activities of garlic [98]. Some researchers have indicated that the extract of garlic shows high scavenging of free radicals and therefore has powerful antioxidant ability [99,100]. Furthermore, other studies have reported that garlic shows antioxidant ability including one research which analyzed this ability using in vivo experiments [101]. Amino acids such as alliin represent one of the important components of garlic [102]. The alliinase enzyme can convert alliin to allicin which is one of the main components of garlic. This chemical compound is responsible for the antimicrobial activity of this herbal medicine [103]. Garlic was demonstrated to exhibit antibacterial activity against a varied range of different bacteria (Gram-positive and Gram-negative) such as Klebsiella, Enterococcus faecalis, Pseudomonas, Salmonella typhi, Proteus, Staphylococcus aureus, and Escherichia coli [104–108]. Some studies have also reported high antimicrobial and antioxidant activities of garlic based on the chemical reaction between allicin and thiol groups of various enzymes [109,110]. This plant also shows antiviral activity against influenza virus (H1N1) [111]

Bioactive chemical constituents:

Bioactive chemical constituents of ginger: Mojani et al have investigated the bioactive constituents of ginger by using high performance liquid chromatography (HPLC). According to their study, the main bioactive constituents are 6- gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8- shogaol and 10-shogaol. Bhargava et al have used gas chromatography and mass spectrometry to identify the active constituents of ginger. Their study revealed that ginger has 40 compounds in methanol extract and 32 compounds in ethanol extract. The important constituents out of these are 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8- shogaol, 10-shogaol, 6-paradol and 8-paradol. [112]

Bioactive chemical constituents of garlic: The constituents of garlic vary with the variation in procedure used for the isolation. In most isolation experiments, garlic was found to be enriched in organosulphur compounds. According to Amagase et al , garlic has various constituent compounds. Some of these are (S)-allylcysteine, alliin, ajoene, alicin, diallyl disulfide, diallyl trisulfide, methylallyl disulfide and vinyldithiins [113]

Bioactive chemical constituents of clove:

Clove contains more than twenty bioactive components as identifies using various chromatographic techniques. The bioactive components of both food and medicinal use found in Clove majorly include polyphenols such as flavonoids, hydroxycinnamic acids, hydroxybenzoic acids, and hydroxyphenyl propenols. Among different polyphenolic compounds, the main compound is eugenol; it is contained in fresh plant material ranges between 9.38-14.65 g/100 g, phenolic acid-like gallic acid is also present in higher concentrations, i.e., around 783.50 mg/100 g fresh weight, hydrolyzable tannins constitutes around 2.38 g/100 g which is determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis . Other minor compounds found are ferulic acid, ellagic acid, caffeic acid, salicylic acid, and flavonoids like quercetin, kaempferol, limonene, ß-pinene, farnesol, 2-heptanone, benzaldehyde, ethyl hexanoate, etc. However, the concentration of various bioactive compounds in the extracted oil varies depending upon the method of extraction from the Clove, These bioactive compounds are shown to be associated with many functional and medicinal health benefits, which will be discussed in further sections.[114]

Safety and Quality Control

WHO Guidelines for Quality Standardized Herbal formulations Standardization and quality control parameters for herbal formulations are based on following fundamental parameters:

1. Quality control of crude drugs material, plant preparations and finished products.
2. Stability assessment and shelf life.
3. Safety assessment; documentation of safety based on experience or toxicological studies.
4. Assessment of efficacy by ethnomedical information and biological activity evaluations.

Quality Control of Crude Material

According to pharmaceutical manufacturers association of U.S. “quality is the sum of all the factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product”[115]. Standardization describes all measures taken during manufacturing process and quality control leads to reproducible quality of particular product. Growing need for standardization and quality control of herbal medicines is recognized by WHO. In policies and checklist on Traditional Medicine(TM) WHO has given emphasis on development of national standards and technical guidelines and methodology for evaluating safety, efficacy and quality of TM. As well as WHO also gives stress on development of national pharmacopoeia and monographs of medicinal plants, cultivation and conservation of medicinal plants to ensure their sustainable use are also prime importance as botanicals are considered [116]. Standardization of botanicals offers many obstacles because synthetic drugs have well defined structure and other analytical parameters as well as reference standard for comparison also established assays and pharmacopoeias. Therefore, quality control is not problematic for synthetic drug [117]. There are several challenges as standardization of herbal product is considered like controversial identity of various plants, deliberated adulteration of plant material, problems in storage and transport, which should be considered [118]. One of the impediments in the acceptance of the herbal products worldwide is the lack of standard quality control profiles. Most of the herbal formulations, especially the classical formulations of traditional medicine, are polyherbal. Each formulation contains 10-20 or more ingredients; a few have even 50-75 ingredients. Many preparations are either liquid or semisolid. For such formulations it is very difficult to establish parameters for quality control. Even official standards are not available. The unique processing methods followed for the manufacture of these drugs turn the single drugs into very complex mixture, from which separation, identification and analysis of the components is a very difficult. In Germany, physicians are required to have some training in herbal medicine. Considerable research on herbal medicine including double-blind, placebo-controlled trials are ongoing. Physicians recommend and patients use herbal medicines extensively. Manufacturers are required to meet standards of purity and pharmaceutical activity. Commission E, which has had oversight of herbal medicines and has determined their safety and efficacy, has published 387 monographs (recently translated into English by the American Botanical Council). Several other European countries also have policies and procedures that allow rational oversight of herbal medicines [119]. In the United States, herbs are used either as dietary supplements, with minimal standards of safety and efficacy, or as drugs, which require expensive and cumbersome testing procedures. Middle-of-the-roadapproach that acknowledges the long history of use of many herbal medicines, examines data from many sources including other countries, insists on strict production standards, and requires absolute safety and a classification of efficacy that may vary from "unproved" for some conditions to "demonstrated" for others. The use of the translated Commission E monographs would be very helpful for patients and physicians.

Conclusion

In both conventional and contemporary medicine, the combined benefits of herbal preparations for improved immune support offer a potential field for study and use. This thorough analysis emphasizes the various ways in which these formulations work to produce their positive benefits, such as cytokine production regulation, antioxidant activity, anti-inflammatory qualities, and antiviral actions.A comprehensive approach to immune health can result from the synergistic interactions between several bioactive substances in herbal mixtures, which can boost immune responses. Crucial herbs like garlic, ginger, and cloves, among others, have shown great promise in boosting immunity and reducing oxidative stress, which will improve general health and wellbeing.Although the encouraging results, it is critical to recognize that more investigation is required to clarify the precise modes of action, ideal dosages, and possible interactions of these herbal compositions. For herbal products to be safe and effective in clinical settings, standardization and quality control are essential.Integrating evidence-based herbal formulations into healthcare practices could be a useful tactic for boosting immune support and optimizing health outcomes as interest in natural medicines continues to expand globally. In order to provide solid proof of these herbal combinations' effectiveness and open the door for their integration into standard treatment regimens, future research should concentrate.

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