An Analysis of Antioxidant and Anti- Microbial Properties of Therapeutic *Kadha Tablet* for Immunity Booster

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Abstract: A unique kind of infectious pneumonia brought on by the SARS coronavirus is known as a severe acute respiratory syndrome. Worldwide efforts are currently being made to tackle the coronavirus sickness, and scientific communities are working tirelessly to provide vaccines. Only a few particular medicinal interventions are available for SARS-CoV-2, nevertheless. We can strengthen our immunity with natural items in addition to other public health measures implemented to prevent this infection. The potential of common spices and herbs as antiviral and immunity-boosting substances has been highlighted in this article. During COVID-19, a questionnaire-based online survey on home remedies was done among a large number of participants (n=531) from diverse nations and age categories (13–68). The study found that 71.8% of people are using kadha to enhance immunity and fight infections. The majority of people (86.1%) believe that kadha has no adverse effects, while 13.9% believe the opposite. A total of 93.6% of respondents believe that spices can improve immunity and help treat coronavirus or other viral infections. For increasing their immunity, most people use chyawanprash, vitamin C, and tulsi drops. As a result, we draw the conclusion from the survey and the literature that spices and herbs are important in the fight against viral infections.

Key Words: —*Immunity Covid-19, antioxidant, anti-Microbial Properties.*

I. INTRODUCTION

Immunity is the state of protection against infectious disease conferred either through an immune response generated by immunization or previous infection or by other non-immunological factors**.** The immune system helps to protect the host from pathogens while minimizing damage to self-tissue **[1].** Immune system is the second line of body defense mechanism. The immune system consists of an intricately linked network of cells, proteins and lymphoid organs which are strategically placed to ensure maximal protection against infection. Immune defenses are normally categorized into the innate immune response, which provides immediate protection against an invading pathogen, and the adaptive or acquired immune response, which takes more time to develop but confers exquisite specificity and long-lasting protection.

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The immune system is made up of good bacteria that live in the gut and defend the human body from a variety of diseases. When the immune system's response is low, weak, or impaired, infections like the corona virus or diseases like diabetes, heart disease, or cancer might thrive **[2].**

Ayurveda is the oldest science of life and health care in the world, its antiquity going back to the ancient Vedas. Its classical ancient knowledge-base has survived up to the modern times through a set of six authentic ancient books consisting of three pre-Christian texts namely Crake, Surtax and Semites of Vagbhatta popularly known as Bhatt ray besides three medieval texts namely Mādhavanidāna, Śārangdhara summit and Bhāvaprakāśa collectively called Laghuttrayī. Immunity is the central focus of modern immunology. Immunity is a biological expression that expresses a state of having abundant biological defences to avoid various disease, infections and other unwanted biological invasion. Ayurveda's widespread knowledge base on preventive care, derives from the concepts of "Dinacharya" - daily regimes and "Ritucharya" - seasonal regimes to maintain healthy life **[3].**

There is no current evidence that any product or practice will contribute to enhanced "immune boosting" protection against COVID-19 **[4].** This lack of evidence has not stopped

wellness gurus, celebrities, and commercial entities from propagating notions of boosting immunity, and messaging of this nature is readily found connected to online portrayals of COVID-19 in the popular press. With the abundance of misinformation circulating online **[5],** this research provides a sense of how immune-boosting discourse is presented on Instagram, one of the world's largest social media platforms. Instagram, the photo- and video-sharing social networking service owned by Facebook, Inc., has over 500 million users active daily **[6].** The onset of clinical disease and its progression to the severe stage may vary between individuals and that depend upon their immune status, and the presence of underlying medical conditions. In general, the typical clinical symptoms include, dry cough (67%), fever (88%), fatigue (38%), myalgias (14.9%), Dyspnoea (18.7%), other symptoms include, headache, sore throat, rhinorrhoea, and gastrointestinal symptoms. Pneumonia is severe manifestation of the infection (7). Epidemiologist says pandemic as a disease that occurs globally or across very broad geographic areas and which cross international borders and has infected large numbers of people **[8]** This requires balance and harmony to work well. Choosing a balanced lifestyle is our first line of defense. Following general recommendations for good health is the single best measure we can take to keep our immune system strong and balanced, obviously. When our immune system is functioning properly, it protects us from environmental threats while also assisting us with safe living techniques such as proper dietary habits. As with every fighting force, a good army of the immune system marches on its heart. Good warriors against the immune system need healthy and daily nutrition **[9]**

1.1 Immunity booster food

The food plays a key role in deciding generally health and immunity (Fig 1). Eating a low-fat, plant-based eating habit may help give the immune system a boost. The immune system depends on WBCs which produce antibodies to battle against microbes, viruses etc. Plant-based diets boost intestinal beneficial bacteria and the general health of the gut micro biome, which accounts for up to 85% of the body's immune system. Excessive consumption of animal foods, on the other hand, depletes the body's healthy bacteria, promotes inflammation, and is the root cause of diabetes, COPD, cardiovascular disease, hepatitis B, cancer, and chronic kidney disease **[1].** Generally, plant-based diets are non-toxic and without any side effects. Various parts of medicinal plants are popular for their antiviral activities and immunity strengthening capacity. In times, when the world is busy fighting deadly corona virus, it is necessary to take extra precautions to keep yourself protected from getting infected. Therefore, the best way to strengthen our immune is to naturally with the help of medicinal plants/herbs. Mother nature has already blessed us with plenty of medicinal plants that help in fighting and curing the vast range of diseases. Ayurveda, the ancient medical science had stated long ago that plant extracts could do a lot to strengthen the body **[10].** Some of the important medicinal plants are as follows that potentially can help in boosting immunity against COVID-19 and other infectious disease: *Drumstick tree (Moringa oleifera), Amla (Phyllanthus emblica), Ashwagandha (Withania somnifera), Giloy/ Guduchi (Tinospora cordifolia), Neem (Azadirachta indica), Garlic (Allium sativum), Tulsi (Ocimum sanctum), Cinnamon (Cinnamomum verum), Turmeric (Curcuma longa), Onion (Allium cepa), Wild carrot (Daucus maritimus), Black pepper (Piper nigrum).*

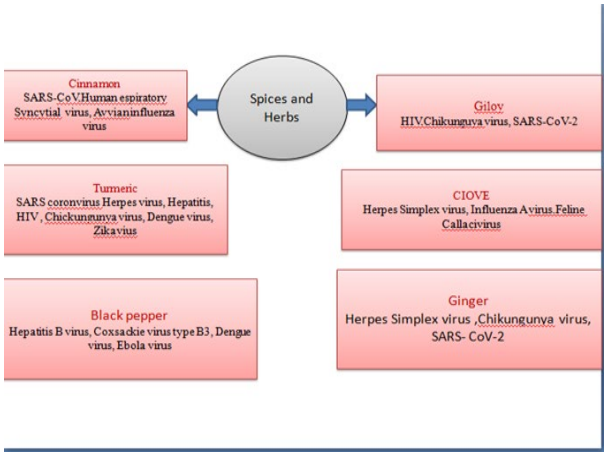


Fig.1. The various types of immunity booster

II. MATERIAL & METHODS

2.1 Point Composite Scoring Test

The Composite scores are calculated from data in multiple variables in order to form reliable and valid measures of latent, theoretical constructs.The variables which are combined to form a composite score should be rele one another. This can be tested through factor analysis and rel analysis. An example of a simple, first-order factor structure; which justify the calculation of three composite scores. To assess the t consistently, absence of defects, color and and overall acceptability product, the products were subjected to sensory evaluation by adopti composite scoring test.

Differently coded samples presented top members one at a time and they were asked to rate their composite so response on the test.

Tool sensory evaluation card is used as a tool for scoring and evaluation.

2.2 Microbiological analysis

2.2.1 Ager diffusion method

Cultivation, maintenance, enrichment, differentiation, tests, and long-term preservation are all possible uses for these medium. Each functional classification is constrained by the formulation and has a limited shelf life. The shelf-life of these culture media refers to the amount of time the medium preserves its chemico-physical properties. Commercially accessible solid growth media on Petri dishes (agar plates) often have a short shelf life, typically ranging from 30 to 90 days. Medium composition (i.e., general-purpose to specialized formulations), sterilization process, storage temperature, exposure to light, and packaging are all important elements that influence total shelf-life and microbiological growth efficiency. Several studies have shown that packed agar plates intended for drug sensitivity testing, isolation, enrichment, or selection have a variable shelf life**.** Bacteria, fungus, and parasites are all examples of microorganisms. Ulisse et al., for example, examined 12 different media types with varying degrees of complexity, packed in shrink-wrap film, stored at 5 °C, and coded for shelf-lives ranging from 3 to 30 days. These media could be given shelf-lives of 90 to 120 days after testing for weight loss (water loss), sterility, pH, and ability to maintain bacterial growth. It is required to support the target microorganism's development and characterisation.

2.3 Test Method

2.3.1 TSS (Total Suspended Solids)

- The refractometer prism surface was cleaned and dried.
- Then, the placed a small amount of sample drops on to the prism of the refractometer
- After look through the eyepiece while pointing the prism in the direction of good light*not directly at the sun
- Focused and took the reading of where the base of the blue color sits on the scale.
- Then, recorded the % Brix

2.4. Total Plate Count Microbial Shelf Life

- Make a dilution series from a sample upto10-4
- Pipetted out 100µl from the appropriate desired dilution series on to the center of the surface of an agar plate.
- Flamed the glass spreader over a lamp.
- Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the petri dish underneath at the same time
- Incubated the plate at 37℃ for 24 hours.
- At the end of the incubation period, selected all of the petri plates containing between 30 and 300 colonies. Plates with more than 300colonies cannot be counted and are designated too many to count (TMTC).
- Calculated the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies multiplied by dilution factor divided by the amount of specimen added to liquified agar.
- This process was done for different time period (day1,3,5) of same sample.

2.4.1. Determination of Colony forming units

- Using aseptic technique, the initial dilution was made by transferring 1 ml of sample to a 9ml sterile peptone water. This is a 1/10 dilution.
- The 10-1 dilution is then shaken by grasping the tube between the palms of both hands and rotating quickly to create a vortex.
- This serves to distribute the bacteria and break up any clumps.
- Immediately after the 10-1 was shaken, uncapped it and aseptically transferred 1ml to a second 9ml peptone water.
- Since this was a 10-1dilution, second blank represents a 10-2dilution of the original sample. It was subsequently diluted upto 10-4dilution.
- Pipetted out 100µl from the appropriate desired dilution series on to the center of the surface of an agar plate.

- Then poured the cooled medium into the plate and then agar-sample mixture are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the table top/platform of LAF cabinet.
- After the pour plates cooled down and the agar was solidified completely, they were inverted and incubated at 37℃ for 24 hours.
- At the end of the incubation period, selected all of the petri plates containing between 30 and 300 colonies. Plates with more than 300colonies cannot be counted and are designated too many to count (TMTC).
- Plates with fewer than 30 colonies are designated too few to count (TFTC). Count the colonies on each plate.
- Calculated the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquified agar.

Number of Bacteria/ml = Number of colonies observed/ amount plated on media*Dilution **factor**

2.5. CFU

Stands for colony forming units, which is a measure of live bacteria or fungi. Unlike a direct microscopic count, which counts all cells alive and dead, CFU estimates viable cells. The development of a viable colony necessitated tremendous growth, and it is impossible to tell whether the colony came from one cell or 1,000 cells at the moment of counting. To account for this uncertainty, the findings are reported as CFU/ ml for liquids and CFU/ g for solids.

2.5.1 Total Suspended Solids (TSS)

Sample filtration of a known volume via 4.7 cm Whatman GF/C glass fiber filters and drying at 103–105 °C were used to estimate TSS. The TSS was calculated using the difference in filter weight before and after filtration.

2.6 Phytochemical Analysis

2.6.1 Determination of Antioxidants:

The free radical scavenging activity of methanol extract of energy bar was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) using the method of Blois (1958)

The free RSA of the energy bar was tested using a 1,1-diphenyl-

- 2-picryl hydrazyl (DPPH) technique. A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of energy bar and methanol solution.
- Four different concentration of energy bar ranging from 10^{-1} to 10^{-4} were prepared for antioxidant analysis in methanol.
- Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants.

% Of antioxidant activity= [(Ac−As) ÷Ac] × 100

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

Fig.2. Antioxidant estimation

III. RESULT & DISCUSSION

3.1 Microbiological Analysis

3.1.1 Test Report

Test: Determination of Colony forming units (CFU/ml). *Method used:* Total Plate Count by Pour Plate Method.

3.1.2 Media Used:

- Total Plate count: Plate Count Agar Media
- TSS: Total suspend solid
- Test Sample: T1

3.1 3. Observation and result

Fig.1. Total Plate count on Plate count agar media

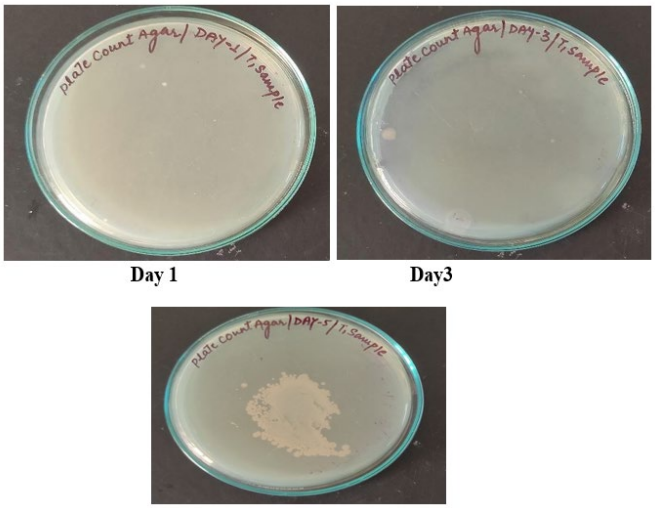


Fig.2. Shelf-life on Plate count media in different time period for sample T1

Fig.3. Measuring TSS (Total Suspended Solids) On Refractometer

Table.1. Total %Brix (T1 Sample)

S.No.	Sample code	% Brix	*Average TSS±SD*
1.	T1	*2%*	*2.33±0.471*
2.	T1	*2%*	
3.	T1	*3%*	

Table.2. Total CFU count

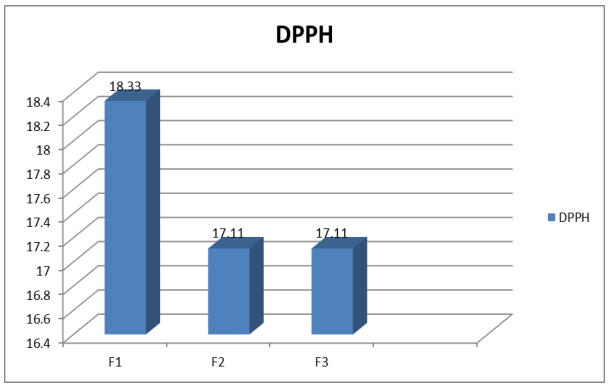
S.No.	Sample code	PCA	
1.	T1	Count	CFU/ml
		65	65×10^{-3}

Table.3. Shelf-life in different time period (T1 sample)

S.No.	Sample code	Day 1		Day 3		Day 5	
1.	T1	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
		3	3×10^{-3}	6	6×10^{-3}	66	66×10^{-3}

***PCA:** Plate count Agar (total plate count); MAM: MacConkey Agar Media (total coliform count); SDA: Sabouraud dextrose Agar (yeast and mold count); Dilution used: 10-4; Sample amount plated: 100 µl; N.A.: Not applicable; TMTC: Too many to count

3.2 Antioxidant analysis of Kadha Tablet



IV. CONCLUSION

In the current pandemic scenario, precautions and boosting immunity are one of the best choices to get away from COVID-19 infection. As per our study, we conclude that the

uses of spices and herbs may play a significant role against viral infections. We have analyzed that cinnamon, black pepper, basil, and turmeric play a vital role against SARS-CoV-2 (COVID-19) as well as other viral infections, which was also supported by some other recent studies. In India, people are using spices as well as herbs from ancient times due to their taste, antiviral, antimicrobial, antioxidant, and immunity-boosting properties. Since ages Indians are habitual of taking these natural products that have conferred immunity in the Indian population, which probably is the major cause for low mortality in India. However, the excessive use of spices and herbs may cause various side effects, namely, acidity in the stomach, heartburn, constipation, diarrhea ulcers in the mouth, high blood pressure, and so on. Therefore, detailed studies about the bioactive compounds present in common Indian herb and spices and their effectiveness and mode of action against lethal viruses need to be explored.

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Conflict of interest:

The authors declare that there is no conflict of interests among them regarding the publication of this paper.

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