

# Formulation and evaluation of immune booster powder

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## Abstract

*Immunity refers to the human body's capacity to detect and combat germs, thereby preventing their potential to cause illness. In recent times, the utilization of medicinal plants for their immune boosting potential have been preferred over pharmaceutical drugs due to their low toxicity and costs. This study is aimed at evaluating the photochemical and the mineral elements in this immune booster formulation. The elemental analysis was conducted using an atomic absorption spectrophotometer. The photochemical screening of the immune booster revealed that the formation contained alkaloids, flavonoids, phenols and saponins while steroids, tannins and glycosides were present. Immune booster formulation showed a good antioxidant activity for ABTS radical (IC) Syrup contains all the herbal drugs which show immunity boosting and health benefits activity, like ashwagandha, tulsi, amla, ginger, fennel and turmeric etc. also contain a main ingredients jaggery which added in liquid form it act as a good immunity booster and base of syrup also. Jaggery is also act as a good preservative to preserve food items.*

**Keywords** *Immunity, Cardiac, cholesterol, immunological cells, antibacterial, tumor; immune system, Beetroot*

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## Introduction

The usage of medicinal plants in recent years has increased because of their user-friendliness, and affordability. Current research findings have also shown that their toxicity level is low as compared other synthetic pharmaceutical counterparts<sup>[1]</sup>. Lately, traditional medicine research has given high priority to immune boosting medicinal plants due to the prevalence of diseases like HIV/AIDS which affect the immune system<sup>[2]</sup>. However, very little information on plants with immune boosting potentials have been documented<sup>[3]</sup>. Existing knowledge has been passed through generations by traditional health practitioners (THPs)<sup>[4]</sup>. *Moringa oleifera* is a plant native to the Indian subcontinent that has become naturalised in tropical and subtropical regions around the world<sup>[5]</sup> and is a fast-growing, drought-resistant tree also known as the drumstick tree, horseradish tree, and ben oil tree or benzolive tree that can be eaten and is extremely safe<sup>[6]</sup>. In common language it's also known as "sahajan"<sup>[7]</sup>. Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals. It is also a very important plant for its medicinal value. Various parts of the plant such as leaves, roots, seeds, bark, fruit, flowers and immature pods etc. as cardiac and circulatory stimulants, possess antitumor antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, cholesterol lowering,

antioxidant, antidiabetic, antibacterial and antifungal<sup>[8]</sup> Recent evidence shows that immunological cells (e.g., lymphocytes) change their responsiveness to signalling from these neurotransmitters and hormones during stress<sup>[9]</sup>. The pro-inflammatory cytokines, interleukin- 1, interleukin-6 and tumor necrosis factor  $\alpha$  are classifications as formal Sleep Regulatory Substances<sup>[10]</sup>. Research models have shown that sleep quality and immune function are closely connected. But there are also sleeve promoting features for several other immune and pro inflammatory cell classes<sup>[11]</sup>. Food and dietary supplements have been used to enhance the health and efficiency of the immune system<sup>[12]</sup>. Synthetic preservatives used in foods may have negative health impacts for hundreds of years<sup>[13]</sup>. The Moringa leaf extract can be used to boost the immune system<sup>[14]</sup>. *Moringa oleifera* is one of the plants that have the antiviral capacity and known to treat various respiratory diseases for decades<sup>[15]</sup>. The antiviral mechanisms are obtained through the inhibition of virus replication<sup>[16]</sup>.

## Materials

### Moringaoleifera

Moringaoleifera is a perennial tree, still considered as among underutilized plant and falls under Moringaceae family. The leaves, flowers and fruits of this plant are associated with high nutritional value of its edible portions pave a way in making this plant more popular as an this plant leaves are a crucial food source for combating protein energy malnutrition due to their rich antioxidant content, providing nutraceuticals and functional components<sup>[17]</sup>

### Beetroot

Beetroot is a rich source of minerals like Magnesium, potassium, iron, copper, and manganese, along with antioxidants and vitamins A B and C. Which **AmlaaA**<sup>[18]</sup>

### Amla

Amla, also known as *Emblica officinalis*, is a member of the small genus *Emblica* (Family Euphorbiaceae). (Amla) has been reported to have strong anti-oxidant properties. Studies are being conducted on the immunomodulatory properties of Amla fruit extracts in immuno-compromised states, with a particular focus on lymphocytes<sup>[19]</sup>

### Jaggery:

Minerals and vitamins (Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B4, Vitamin B5, Vitamin B6, Vitamin B7, Vitamin B8, Vitamin B9, Vitamin B12, Vitamin C, Vitamin D2, and Vitamin E) are considered beneficial sources of food in the United States in order to address malnutrition and immune issues.11 Drinking herbal drink contains jaggery is very beneficial to increase immunity.<sup>[20]</sup>

### Ashwagandha:

Ashwagandha (*Withaniasomnifera* or WS) that's referred to with inside the Rasayana organization of medicinal drugs with inside the Ayurveda culture of India has been notably investigated as an immunomodulatory agent<sup>[21]</sup>

### Cinnamon :

Cinnamon (*Cinnamomumzeylanicum*, and Cinnamon cassia), the eternal tree of tropical medicine, belongs to the Lauraceae family.14 Cinnamon consists of a variety of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils. It is to being used as a spice and flavoring agent<sup>[22]</sup>

### Flaxseed :

Flaxseed, known as *Linum usitatissimum*, is gaining importance as a functional food ingredient due to its high content of  $\alpha$ -linolenic acid, lignans, and fiber.<sup>[23]</sup>

### Sesame seed :

Sesame (*Sesamum indicum* L.) is a member of Pedaliaceae family and *Sesamum* genus. Sesame seed is a rich source of unsaturated fatty acids (83%-90%), mainly linoleic acid(37%-47%), followed by oleic acid (35%43%), stearic acid (5%-10%), palmitic acid (9%-11%) and trace amount of linolenic acid also present in it (Pathak et al., 2017). Besides macronutrients, sesame has also considerable amount of lignans (sesamin, sesamol)<sup>[24]</sup>

## Methodology

### Formulation of leaves powder Drying

#### of leaves

- **Collection:** : The leaves were collected from the university campus
- **Sorting:** Fresh, green undamaged leaves were collected to produce the best quality powder.
- **Cleaning and washing:** The stalk of the leaves was cut from the main branches and were washed 3-4 times with plenty of water to remove all the adhering dust, and dirt particles.
- **Blanching and Drying:** leaves were immersed in boiling water at 100 o

C for 5 minutes and then it was immersed in cold water for 2 minutes. Blanched leaves were dried in neat and clean dust free dark shadow place at temperature ranged from 25-30 oC. The leaves were completely dried in 2438 hours. Dried them till leaves became brittle and crushed easily.

**Grinding of leaves:** Dried leaves were ground into fine powder. leaves powder were stored in airtight containers protected from humidity, heat, and light to avoid the growth of molds at room temperature.

**Storage :** Moringaoleifera leaves powder were stored in airtight containers protected from humidity, heat, and light to avoid the growth of molds at room temperature.

Sr. no	Ingredients	Quantity F1(for 50gm)	Quantity F1(for 50gm)	Quantity F1(for 50gm)	Quantity F1(for 50gm)	Quantity F1(for 50gm)	Use
1	Amla	10	11	9	8	7	Improves Immunity, Reduces stress
2	Beet root	8	9	6.5	7	6.5	Reducing inflammation
3	Jaggry powder	q.s.	6	7	q.s.	q.s.	Immune system
4	Moringa	15	14	16	17	15.5	Diabetes, inflammation
5	Ashwagandha	3	2	4	5	6	Reduce stress and anxiety
6	Sesame seed	3	2	3.5	1	4	They used as an ingredient in lubricant, medicines
7	Cinnamon	3	2.5	2	1.5	2.5	Antioxidants
8	Flax seed	3	3.5	2	2.5	1.5	Improve digestive health

## Evaluation Parameter

### PH

1gm of immunobuster sample was taken in 100 ml dry beaker, 100 ml water is added to it and set aside for 2 hours. The PH of sample was measured by using digital type PH meter. The PH of sample was found to be 5.9

### Determination of moisture content:

A glass-stopper and shallow weighing bottle were used to accurately weigh 2gm of a sample, which was then covered and covered to ensure accurate measurements. Then loaded bottle was kept in an oven and was removed. The sample was dried to constant weight. After drying it was collected to room temperature in a desiccators. weighed and loss on drying was calculated in terms of percent w/w.

### Determination of total ash:

Accurately weighed 2gm of sample was taken in a tared silica dish and incinerated at a temperature not exceeding 450 degree celcius until free from carbon, cooled and weight was taken. The percentage of ash was calculated.

W2-W1.

### Determination of water-soluble ash:

2gm of sample and boiled for 5 minutes with 25ml of water, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 degree celcius and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated.

W2-W1

### Determination of Acid-insolubleash:

Take 2gm of sample and boiled for 5 minutes with 25ml of 2M hydrochloric acid, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited cooled in a desiccators and weighed. The percentage of acid- insoluble ash was calculated.

W2-W1

### Loss on drying:

Loss on drying is widely used test method to determine the moisture content of sample. Loss on drying was determined by placing 2gm of immune booster powder in petri dish in hot air oven and taking weight of sample after half hour of interval and weight loss by sample was reported.

Formula for loss on drying

$$\text{LOD} = \frac{W1 - W2}{W1} \times 100$$

W1=Empty petri dish weight

W2=Sample petri dish weight

W3=Weight after drying **Solubility**

For solubility test 1gm of sample of immune booster powder was dissolved in sufficient amount of water.

### Angle of repose

When a powder is allowed to fall on a flat surface from a funnel positioned at a certain height, the funnel is gradually moved upward in order to maintain a fixed height between the powder tip and the bottom of the funnel. The angle produced by the powder on the surface is the angle of repose

$$\text{Angle of repose } \theta = \tan^{-1} h/r$$

Where,

h=height

r=radius

### Evaluation of antioxidant activity

#### Scavenging activity of hydrogen peroxide

Hydrogen peroxide is used as a free radical to determine the antioxidant activities of natural compounds. The discoloration of the test compound is attributed to its hydrogen-donating ability. Hydrogen peroxide being a commercially stable radical, soluble in methanol gives a violet solution, which upon contact with an antioxidant, changes to a corresponding yellow colour (24). Two gram of the immune booster sample (0–5 mg/mL CH<sub>3</sub>OH) were added to 2 mL of hydrogen peroxide (0.4 mM hydrogen peroxide in CH<sub>3</sub>OH) respectively. The mixtures were vortexed thoroughly and incubated at room temperature in the dark for 30 min. The absorbance of the reaction mixtures was measured at 230 nm using spectrophotometer. Gallic acid and Ascorbic acid used as standard drugs.

#### 1. Organoleptic Evaluation:

Sr. no	Parameter	Observation				
		F1	F2	F3	F4	F5
1	Appearance	Powder	Powder	Powder	Powder	Powder
2	Texture	Fine	Fine	Fine	Fine	Fine
3	Colour	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown
4	Odour	Sweet	Sweet	Sweet	Sweet	Sweet

Table : Organoleptic Evaluation

#### 2 . Physical Parameter and physicochemical Evaluation:

Sr. no	Parameters	Observation				
		F1	F2	F3	F4	F5
1	PH	5.9	5.4	6.0	6.2	5.2
2	Ash value	0.14% w/w	0.13 % w/w	0.15% w/w	0.11% w/w	0.13% w/w
3	Loss on drying	0.14%	0.10%	0.086%	0.29%	0.10%
4	Acid insoluble Ash	0.11%	0.08%	0.07%	0.09%	0.04%
5	Water soluble Ash	0.06%	0.07%	0.06%	0.04%	0.08%

6	Angle of repose	8.2°c	7.5°c	6.4°c	7.8°c	5.2°c
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### Qualitative phytochemical tests

#### Ellagic test for phenols

2 ml of immune booster sample was treated with four drops of 5 % (weight/volume) glacial acetic acid followed by four drops of 5 % (w/v) NaNO<sub>2</sub> solution. The mixture was shaken for 5 minutes. The appearance of muddy brown precipitate indicates the presence of phenols.<sup>19</sup>

#### Ferric Chloride test for flavonoids

About 5 drops of 10 % ferric chloride solution were added to 2 ml of the immune booster sample. Colour change to green-blue would indicate the presence of phenolic hydroxyl group.<sup>21</sup>

#### Sulphuric acid test for glycosides

To 2.5 ml of immune booster sample, 2 ml glacial acetic acid was added. This was followed by addition of one drop of 5 % FeCl<sub>3</sub>. The solution was then acidified by dropwise addition of concentrated H<sub>2</sub>SO<sub>4</sub>. Appearance of brown ring at liquid interface would indicate the presence of glycosides <sup>21</sup>

#### Wagner's test for alkaloids

5 drops of Wagner's reagent were added to 2 ml of immune booster sample. The mixture was warmed for one minute. Formation of reddish brown precipitate indicates the presence of alkaloids.<sup>23</sup>

#### Molisch' test for carbohydrates

Small amount of sample was treated with 2-3 drops of 1% alcoholic  $\alpha$  naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

#### Fehlings test for carbohydrates

The small amount of sample was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained the presence of free reducing sugars.

#### Millon's test for protein and amino acids

Small amount of sample was heated with Millon reagent. White precipitate turned red on heating indicate the presence of proteins.

### Conclusion

In conclusion, keeping our immune systems healthy is important for our general health as they play a major part in defending our bodies against illnesses. Maintaining your health and boosting your immune system can be achieved through adopting a healthy lifestyle. Although there are allopathic medications that can fight oxidative stress and thereby boost immunity, it is essential to look for a different option due to the side effects and high price of these medications. In terms of natural drug development, ayurvedic medicines have a promising profile. Herbs are expected to serve as the main ingredient in the creation of an affordable, efficient, and safe immunomodulatory drug. A good proportion of nutrition (fat, fiber, carbohydrates), Minerals (P, Mg, Mn, Se) and Vitamins (thiamin, riboflavin, vit k) is achieved when compared to nutritional value of standard food product (Protein X). From the study we can conclude that it is possible to find right combination of organic food product, medicinal plants and probiotics as food supplement. In day to day busy and stress life most of the population are getting inadequate vitamin, mineral and other plant active compounds.

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