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FORMULATION AND EVALUATION OF A POLYHERBAL POWDER FOR IMMUNITY ENHANCEMENT USING MORINGA OLEIFERA, TINOSPORA CORDIFOLIA, AND CITRUS LIMON

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Abstract:
The present investigation was carried out to formulate and evaluate a polyherbal powder intended for immunity enhancement using Moringa oleifera, Tinospora cordifolia, and Citrus limon. The selected plant materials are well known for their nutritional, antioxidant, and health-promoting properties.
The herbal powder was prepared by drying, pulverizing, sieving, and blending the plant materials in appropriate proportions. The developed formulations were evaluated for organoleptic characteristics, physicochemical parameters, phytochemical constituents, flow properties, stability, and antioxidant activity. Preliminary phytochemical analysis confirmed the presence of important bioactive compounds including flavonoids, alkaloids, tannins, saponins, and phenolic constituents. Antioxidant activity was determined using the DPPH radical scavenging assay, where formulation F1 exhibited the highest scavenging activity of 82%.
The prepared polyherbal formulation showed satisfactory stability, acceptable physicochemical properties, and appreciable antioxidant potential. The synergistic combination of Moringa, Giloy, and Lemon may contribute to improved immune-supportive activity and reduction of oxidative stress. The findings suggest that the developed formulation can be considered a promising, safe, and cost-effective natural supplement for immunity enhancement and overall wellness.

Keywords: *Polyherbal powder, Immunity enhancement, Moringa oleifera, Tinospora cordifolia, Citrus limon, Antioxidant activity, Phytochemical screening*

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INTRODUCTION :

Increasing interest in natural healthcare products has encouraged the use of herbal formulations for maintaining health and improving immunity (7)."
Medicinal plants are rich sources of vitamins, minerals, flavonoids, and phenolic compounds that contribute to various therapeutic activities (4)."
Moringa oleifera is widely recognized for its nutritional value and antioxidant potential due to the presence of bioactive phytoconstituents (1)."
Tinospora cordifolia has traditionally been used in Ayurveda for its immunomodulatory and health-supportive properties (6)."

Citrus fruits such as Citrus limon contain Vitamin C and flavonoids that may help protect cells from oxidative damage (3)."

Free radical-induced oxidative stress is associated with several physiological disorders and impaired immune response(5)."

Proper evaluation of herbal formulations is important to ensure their quality, stability, and therapeutic suitability(2)."

Therefore, the present investigation was undertaken to formulate and evaluate a polyherbal powder for immunity enhancement using selected medicinal plant materials.

Aim and Objectives

- **Aim :** To formulate and evaluate a polyherbal powder containing *Moringa oleifera*, *Tinospora cordifolia*, and *Citrus limon* for its antioxidant and immunity-supportive potential.
- **Objectives**
  1. To develop a polyherbal powder formulation using selected medicinal plant materials.
  2. To evaluate the organoleptic and physicochemical characteristics of the prepared formulation.
  3. To identify major phytochemical constituents such as flavonoids, alkaloids, tannins, and saponins through preliminary phytochemical screening.
  4. To assess the antioxidant activity of the formulation using the DPPH radical scavenging assay.
  5. To compare the antioxidant potential of different formulations and determine the optimized formulation.
  6. To study the stability and suitability of the developed formulation for herbal health applications.

Materials :

Figure 1. Moringa oleifera leaf powder.

Moringa *(Moringa oleifera*) is a fast-growing medicinal plant commonly known as the drumstick tree. It is widely used in Ayurveda due to its high nutritional value and therapeutic properties. *Moringa oleifera* leaf powder, *Tinospora cordifolia* stem powder, and *Citrus limon* peel powder were used for the preparation of the polyherbal formulation. All plant materials were collected from local sources and authenticated based on their morphological characteristics. The collected materials were cleaned, shade dried, pulverized, and passed through sieve no. 60 to obtain uniform powder. All reagents and chemicals used during the study were of analytical grade.

1. **Classification**

- Kingdom : Plantae
- Family : Moringaceae
- Genus : Moringa
- Species : Moringa oleifera

➢ **Parts Used**

1. Leaves (most commonly used)
2. Pods (Drumsticks)
3. Seeds
4. Flowers

- **Nutritional Composition**
  Moringa leaves are highly nutritious and contain:
- Vitamins: A, B-complex, C, E
- Minerals: Calcium, Iron, Potassium
- Proteins & Amino acids Antioxidants : Flavonoids, Polyphenols

Figure2.Tinospora cordifolia stem powder.

Giloy (Tinospora cordifolia) is a well-known medicinal plant in Ayurveda, often called "Guduchi" or "Amrita" (meaning "root of immortality"). It is famous for its powerful immunity-boosting and disease-fighting properties. (6)

➢ **Classification**

- Kingdom : Plantae
- Family : Menispermaceae
- Genus : Tinospora
- Species : Tinospora cordifolia

Parts Used

1. Stem (most commonly used)
2. Leaves
3. Roots

Giloy contains many bioactive compounds such as: Alkaloids, Glycosides, Steroids, Diterpenoid lactones

Lemon (Citrus limon) is a widely used citrus fruit known for its high Vitamin C content and refreshing taste. It is commonly used in herbal formulations to improve immunity and add flavor.

➢ **Classification**

- Kingdom: Plantae
- Family: Rutaceae
- Genus: Citrus
- Species: Citrus limon

- Parts Used
- Fruit (juice and pulp)
- Peel (used in powder form)
- Seeds (rarely used)

- Chemical Constituents Lemon contains:
- Vitamin C (Ascorbic acid)
- Citric acid
- Flavonoids
- Essential oils (Limonene)

These compounds provide antioxidant and antimicrobial properties. (3)

Figure 3 Citrus lemon peel powder.

IMMUNITY BOOSTER POWDER FORMULATION (Per 5g Dose
Table No.1

Sr. No	Ingredient	Scientific Name	Quantity (g)	Function
1	Moringa powder	Moringa oleifera	2.0	Immunity booster
2	Giloy powder	Tinospora cordifolia	2.0	Immunomodulator
3	Lemon powder	Citrus limon	0.5	Vitamin C source
4	Excipients (optional)	-	0.5	Flavor improvement
	Total		5g	

Method :

1. **Collection of Raw Materials**

Collect fresh and healthy Moringa leaves, Giloy stems, and Lemon peels.

2. Cleaning

Wash all materials with clean water to remove dust and impurities.

3. Drying

Dry under shade (not direct sunlight)

Continue drying until materials become completely moisture-free

4. Size Reduction (Grinding) Use grinder or mortar & pestle

Convert dried materials into fine powder

5. Sieving

Pass powder through sieve (e.g., #60 mesh) Obtain uniform particle size

6. Weighing

a) Moringa = 2 g
b) Giloy = 2 g
c) Lemon = 0.5 g
d) Excipients (optional) = 0.5 g

7. Mixing

Mix all powders thoroughly

Ensure uniform distribution of ingredients

8. Packaging & Storage

Store in airtight container.

Keep in cool and dry place

"The formulated powder was evaluated using the following parameters:"

- ***Evaluation of powder***

Figure 4 Prepared polyherbal Immunity Enhancement powder.

The formulation exhibited a characteristic herbal odor, greenish-brown appearance, and fine texture suitable for oral administration.

2. Physicochemical Evaluation

Table no. 2

Parameter	Method	Ideal Result
Moisture content	Loss on drying	Below 10%
pH	pH meter	5.5-7
Ash value	Incineration	Low (purity check)
Bulk Density	Measuring cylinder	Uniform packing
Tapped Density	Tapping method	Indicates flow property

The physicochemical parameters were found to be within acceptable limits, indicating good quality of the formulation.

4. Phytochemical Screening

Table no.3

Compound	Test	Result
Alkaloids	Dragendorff's test	Present
Flavonoids	Shinoda test	Present
Tannins	Ferric chloride test	Present
Saponins	Foam test	Present

These compounds are responsible for immunity boosting activity.

These phytoconstituents are responsible for the therapeutic and immunity boosting properties of the formulation.

4. Solubility Test

- Slightly soluble in water
- Better soluble in warm water
- Forms suspension (not fully dissolved).

5. Stability Evaluation

The developed polyherbal formulation was evaluated for physical stability under normal storage conditions. Parameters such as color, odor, texture, and appearance were observed during the study period. No significant variation was noted in the evaluated parameters, and the formulation remained free-flowing without evidence of caking or moisture absorption. These observations indicate satisfactory stability of the prepared herbal powder formulation during storage.

Table no. 4

Parameter	Initial Observation	After stability study
Colour	Greenish brown	No significant change
Odour	Characteristic herbal odour	Retained characteristic odour
Appearance	Free flowing powder	No caking observed
Texture	Fine powder	No significant change

6. Antioxidant Activity (Optional Advanced)

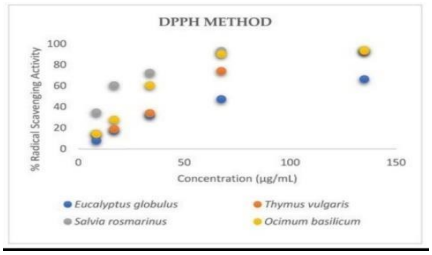

Figure 6 DPPH Radical Scavenging Activity of the Formulation.

➢ **DPPH assay method**

- Shows free radical scavenging activity
- Confirms immunity boosting potential.
- The antioxidant activity confirms the free radical scavenging potential of the formulation.

Table no.5

Parameter	Observation
Test appearance	Light yellow to pale brown solution observed.
Colour change	Gradual reduction in colour intensity noted
Activity	Formulation showed noticeable antioxidant activity.
Inference	Presence of natural antioxidant constituents confirmed.

Result
"The formulated polyherbal powder exhibited appreciable antioxidant activity due to the presence of bioactive phytoconstituents from herbal ingredients."

8.Flow Property Evaluation

Figure 7 Flow Property Evaluation of polyherbal powder

Table no. 6

Parameter	Result
Angle of repose	Good flow
Carr's Index	Acceptable
Hausner ratio	<1.25

Result: The prepared polyherbal powder exhibited satisfactory flow properties with good handling and packing characteristics.

The formulation showed satisfactory flow properties suitable for handling and packaging (Kokate et al.).

Extract Preparation

Overall, the evaluation results indicate that the formulated herbal powder possesses good physicochemical properties, stability, and biological activity, making it suitable as a natural immunity booster.

Tannins: Gelatin Test Preparation of Extract :
Extract the plant sample in water or an appropriate solvent.

Addition of Gelatin Solution:
Prepare a 1% gelatin solution (often containing a bit of sodium chloride to aid precipitation). Add an equal volume of gelatin solution to the plant extract.

Observation:
Formation of white precipitate indicates the presence of tannins.

Result : The gelatin test confirmed the presence of tannins in the prepared polyherbal extract.

Saponins: Froth Test

Figure 8 Froth Test for Detection of Saponin.

Boil or shake the plant material with water. Filter the extract to obtain a clear solution. **Test:**
Shake 2–3 ml of the extract vigorously in a test tube for 2–3 minutes.

Observation: Persistent froth or foam (lasting for at least 10 minutes) indicates the presence of saponins.

Result: The froth formation test confirmed the presence of saponins in the polyherbal formulation.

Antioxidant Assays

A) DPPH Radical Scavenging Assay

Principle: DPPH solution changes color from purple to yellow in presence of antioxidants.
Prepare 0.1 mL DPPH solution in methanol.
Add 1 mL of sample extract to 2 mL of DPPH solution. Incubate for 30 min in dark.
Measure absorbance at 517 nm. Calculate % scavenging activity:

b) Reducing Power Assay

Measures electron-donating capacity of antioxidants.

Procedure:
Mix sample extract with phosphate buffer and potassium ferricyanide. Incubate at 50°C for 20 min.
Add trichloroacetic acid, centrifuge, mix supernatant with $FeCl_3$. Measure absorbance at 700 nm.
'Increased absorbance indicate higher reducing powder."

Summary of Formulation Evaluation

Formulation	Moisture(%)	Ash(%)	pH	DPPH Activity(%)	Taste Acceptability
F1	5.2	6.8	6.1	82%	High
F2	5.5	7.0	6.0	75%	Moderate
F3	5.3	6.9	6.2	78%	High

- **DPPH Radical Scavenging Activity of Polyherbal Formulations**

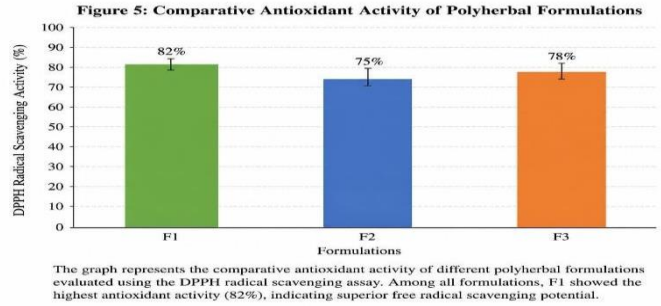

Figure 9 Comparative Antioxidant Activity of Polyherbal Formulation

F1 showed the highest antioxidant activity compared to F2 and F3 formulations.
The comparative graph demonstrates the antioxidant potential of different polyherbal powder formulations evaluated by the DPPH radical scavenging method. Among all formulations, F1 exhibited the highest antioxidant activity with 82% scavenging effect, followed by F3 (78%) and F2 (75%). The increased antioxidant activity may be attributed to the presence of flavonoids, phenolic compounds, and Vitamin C in the selected herbal ingredients. These phytoconstituents are known for their ability to neutralize free radicals and reduce oxidative stress, thereby supporting immune function and overall health (Anwar et al., 2007; Saini et al., 2016). The findings suggest that the developed formulation possesses significant antioxidant potential and may serve as an effective natural immunity-enhancing supplement.

➢ RESULTS AND DISCUSSION:

Prepared polyherbal powder formulations containing Moringa oleifera, Tinospora cordifolia, and Citrus limon were evaluated for organoleptic characteristics, physicochemical properties, phytochemical constituents, antioxidant activity, flow behavior, and stability. The obtained findings indicated satisfactory quality and promising antioxidant potential of the developed formulation.

Organoleptic evaluation revealed that the formulation possessed a greenish-brown appearance with a characteristic herbal odor and mild citrus flavor. The powder showed a fine and uniform texture, indicating proper processing and blending of the herbal ingredients. The slightly bitter taste observed in the formulation was mainly attributed to the presence of Giloy powder.

Physicochemical evaluation included parameters such as moisture content, ash value, pH, bulk density, tapped density, Carr's index, Hausner ratio, and angle of repose. All evaluated parameters were found within acceptable limits. Low moisture content may help improve the storage stability of the formulation by reducing the possibility of microbial growth. The near-neutral pH suggests suitability for oral administration, while the observed flow properties indicate good handling and packaging characteristics of the powder blend. (2)

Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds in the developed formulation. These bioactive constituents are known for their antioxidant and therapeutic activities. Phenolic compounds and flavonoids, in particular, may contribute to free radical scavenging activity and protection against oxidative stress-related cellular damage. (4)

The antioxidant potential of the prepared formulations was determined using the DPPH radical scavenging assay, which is commonly used to evaluate antioxidant activity in herbal preparations (Singleton et al., 1999). Among all tested formulations, F1 exhibited the highest radical scavenging activity (82%), followed by F3 (78%) and F2 (75%). The enhanced antioxidant effect observed in formulation F1 may be associated with the combined activity of phytoconstituents present in Moringa, Giloy, and Lemon. These herbal ingredients are rich in antioxidants and micronutrients that may support immune function and help reduce oxidative stress. (1)

The findings obtained in the present investigation are consistent with previous reports describing the immunomodulatory and antioxidant potential of Tinospora cordifolia and other medicinal plants used in traditional healthcare systems. (6) The observed antioxidant activity suggests that the developed polyherbal formulation may provide supportive benefits for maintaining general health and immune function.

Stability studies demonstrated that the formulation remained physically stable throughout the storage period. No significant changes were observed in color, odor, texture, or appearance, and the powder remained free-flowing without caking or moisture absorption. These observations indicate satisfactory stability of the prepared formulation under normal storage conditions (ICH Guidelines, 2005).

Overall, the developed polyherbal powder exhibited acceptable physicochemical characteristics, appreciable antioxidant activity, and satisfactory stability. The formulation may therefore serve as a promising natural supplement for immunity enhancement and health support.

➢ *Limitation*

"The present study was limited to preliminary phytochemical screening and in vitro antioxidant evaluation of the developed polyherbal formulation under laboratory conditions. Further pharmacological, toxicological, and clinical studies are required to establish the long-term safety, efficacy, and therapeutic potential of the formulation for immunity enhancement."

➢ CONCLUSION:

The present study successfully formulated and evaluated a polyherbal powder containing Moringa oleifera, Tinospora cordifolia, and Citrus limon for immunity enhancement and antioxidant potential.

The developed formulation exhibited satisfactory organoleptic and physicochemical characteristics along with good stability during the study period. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which are known for their therapeutic and antioxidant properties.

The antioxidant evaluation performed using the DPPH radical scavenging assay demonstrated appreciable free radical scavenging activity, with formulation F1 showing the highest antioxidant potential among the tested formulations. The observed activity may be attributed to the synergistic effect of phytoconstituents present in the selected herbal ingredients. The findings suggest that the developed polyherbal formulation may serve as a promising natural supplement for supporting immune function and reducing oxidative stress.

Overall, the prepared formulation can be considered a safe, economical, and plant-based approach for general health support and immunity enhancement. However, further pharmacological, toxicological, and clinical studies are recommended to establish its long-term safety, efficacy, and therapeutic applications. (1,6,4)

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