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Novel Formulation and evaluation of health immunity booster capsule (Prevention of various disease) for Spirulina with Ashwagandha, Beetroot and Apricot

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ABSTRACT

In recent times, the importance of maintaining a robust immune system has become increasingly evident. The present study aims to formulate and evaluate a novel health immunity booster capsule leveraging the synergistic effects of Spirulina with Ashwagandha, Beetroot, and Apricot. Spirulina is a blue-green algae, is renowned for its rich nutritional profile, including vitamins, minerals, proteins, and antioxidants, which are pivotal for immune function. Ashwagandha, an adaptogenic herb, has been traditionally used in Ayurveda for its immunomodulatory properties. Beetroot, abundant in vitamins, minerals, and phytochemicals, complements the immune-boosting potential of Spirulina and Ashwagandha. Apricot, a nutrient-dense fruit, adds additional vitamins and antioxidants, enhancing the overall efficacy of the formulation. The formulation process involves carefully selecting and proportionately combining Spirulina powder, Ashwagandha extract, Beetroot extract, and Apricot powder to optimize their synergistic effects. Various parameters such as particle size, bulk density, flow properties, and compressibility are evaluated to ensure the uniformity and stability of the capsule formulation. Additionally, compatibility studies are conducted to assess any potential interactions between the ingredients that may affect the quality and efficacy of the final product. The evaluation of the health immunity booster capsule encompasses both in vitro and in vivo studies. In vitro assays include antioxidant activity assessment using DPPH and ABTS assays to evaluate the scavenging capacity of free radicals, while cell culture studies are conducted to investigate the immunomodulatory effects of the formulation on immune cells

such as macrophages and lymphocytes. Furthermore, in vivo studies are performed on animal models to assess the safety, bioavailability, and efficacy of the capsule in enhancing immune response and protecting against various diseases. The results obtained from the formulation and evaluation studies provide valuable insights into the potential health benefits of the Spirulina-based immunity booster capsule. The synergistic combination of Spirulina with Ashwagandha, Beetroot, and Apricot demonstrates promising immune-boosting properties, paving the way for the development of natural and effective strategies for preventing and managing diseases. Future research directions may include clinical trials to validate the efficacy of the capsule in human subjects and explore its therapeutic applications in immunocompromised individuals or those at risk of infectious diseases. In conclusion, the formulation and evaluation of the health immunity booster capsule offer a holistic approach to enhancing immune function and promoting overall health and well-being. By harnessing the power of nature's ingredients, this novel formulation holds great promise in supporting immune health and combating the challenges posed by various diseases.

KEYWORDS : Spirulina , Ashwagandha, Beetroot, Apricot , Immunity Booster etc.

Introduction:-

Spirulina

In the early 1960s, Spirulina, a filamentous blue-green (cyanobacteria) algae attracted the interest of researchers. The biochemical components supply the marketing value To Spirulina. Spirulina is especially rich in protein of which Amount to 60-70 percent of its dry weight. Spirulina contains up To 20 percent of phycocyanin, a water-soluble blue pigment [1] And green pigment chlorophyll a. Spirulina contains a relatively High content of cyanocobalamin (vitamin B12). It was also found To contain β-carotene, provitamin A, vitamin C and E (Table 1). Spirulina is the source of essential fatty acid, γ-linolenic Acid (GLA) claimed to have medicinal properties. Spirulina has Also minerals, iron, calcium, chromium, copper, magnesium, Manganese, phosphorus, potassium, sodium and zinc [2].

Fig 1. Spirulina Powder

Nutrients such as phosphorus and nitrogen present in agro- Industrial effluents as well as in domestic wastewater may cause Serious eutrophication in any water body. But these nutrients Can be used to increase

plant growth, such as phytoplankton, Which can be utilized as natural fish food or for pharmaceutical Purpose. Spirulina is one of the most promising microalgae for Culture due to its high nutritional values [3]. Although many Studies have been carried out about the physiology of Spirulina In microalgal biotechnology, the studies about the genetic Isn't enough. Genetic of cyanobacteria was known generally, However, there is need for new improved strains for particular Aims and methods gene transfer system on Spirulina.

Ashwagandha

Ashwagandha (Withania somnifera, fam. Solanaceae) is commonly known as "Indian Winter cherry" or "Indian Ginseng". It is one of the most important herb of Ayurveda (the traditional system of medicine in India) used for millennia as a Rasayana for its wide ranging health benefits. Rasayana is described as an herbal or metallic preparation that promotes a youthful state of physical and mental health and expands happiness. These types of remedies are given to small children as tonics, and are also taken by the middle-aged and elderly to increase longevity. Among the ayurvedic Rasayana herbs, Ashwagandha holds the most prominent place. It is known as "Sattvic Kapha Rasayana" Herb (Changhadi, 1938). Most of the Rasayana herbs are adaptogen / anti-stress agents.

Fig 2. Ashwagandha Powder

Ashwagandha is commonly available as a churna, a fine sieved powder that can be mixed with water, ghee (clarified butter) or honey. It enhances the function of the brain and nervous system and improves the memory. It improves the function of the reproductive system promoting a healthy sexual and reproductive balance. Being a powerful adaptogen, it enhances the body's resilience to stress. Ashwagandha improves the body's defense against disease by improving the cell-mediated immunity. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals.(4)

Beetroot:-

Beta vulgaris (beet) is a species of flowering plant in the subfamily Betoideae of the family Amaranthaceae. Economically, it is the most important crop of the large order Caryophyllales.(5)

Fig 3. Beetroot Powder

It has several cultivar groups: the sugar beet, of greatest importance to produce table sugar; the root vegetable known as the beetroot or garden beet; the leaf vegetable known as chard or spinach beet or silverbeet; and mangelwurzel, which is a fodder crop. Three subspecies are typically recognized. All cultivars, despite their quite different morphologies, fall into the subspecies Beta vulgaris subsp. Vulgaris. The wild ancestor of the cultivated beets is the sea beet (Beta vulgaris subsp. Maritime).

Apricot:-

Apricot (Prunus armeniaca L.) belongs to family Rosaceae. In angiosperms, Rosaceae is one of the largest families having about 3,400 species including almonds, peaches, apples, plums, cherries and berries, distributed throughout the northern temperate regions of the globe. Apricot has been named by Romans most probably from the mixed accent of two words “praecocia” from Latin meaning “early matured”, or “albarquq” from Arabic, meaning short ripening period. It is a temperate fruit and grown in climates with well-differentiated seasons. It requires a fairly cold winter and moderately high temperatures in the spring and early summer (Ahmadi et al., 2008; Guclu et al. 2009) [6]. The apricot tree is deciduous and needs a relatively cold winter for proper dormancy and flower bud development (400–600 hours below 7.2 °C during winter). The cultivation of apricots is not suitable in areas with a subtropical climate. Botanically, apricots are drupes like peaches, plums, cherries and mangoes in which the outer fleshy part (exocarp and mesocarp) surrounds a hard stone (endocarp) with a seed inside. Fruit color ranges between orange to orange red and some cultivars are cream white to greenish white (Ruiz et al. 2008; Riu-Aumatell et al. 2009) [7,8].

Fig 4. Apricot Powder

Apricot originated in China and Central Asia (Yuan et al., 2007) [9] and has been cultivated in china since 2000BC. It gradually made its way through the Persian Empire into the Mediterranean, where they were best adapted. This fruit has also been grown in mountainous slopes of Asia and Europe for thousands of years. Presently, the main apricot cultivation regions include a strip stretching from Turkey through Iran, the Himalayas, Hindukush to China and Japan. However, the largest production of world apricot is supplied from the Mediterranean countries (Leccese et al. 2007) [10]. According to FAO statistics (2010), the world largest producers are Turkey and Iran accounting for 21.6% and 14.7% of world apricot production respectively, followed by Pakistan, Uzbekistan, Italy, Algeria, Japan, Morocco, Egypt and Spain.

Nutritive composition of Spirulina:-

Nutritional value per 100 g (3.5 oz) [11]

Energy	**1,213 KJ (290kcal)**
Carbohydrates	**23.9 gm**
Sugar	3.1 gm
Dietary fibre	3.6 gm
Fat	**7.72 gm**
saturated	2.65 gm
Monounsaturated	0.675 gm
polyunsaturated	2.08 gm
Protein	**57.47 gm**
Tryptophan	0.929 gm
Threonin	2.97 gm
Isoleucine	3.025 gm
leucine	4.947 gm
Lysine	3.025 gm
Methionine	1.149gm
Cystine	0.662gm
phenylalanine	2.777gm
Tyrosine	2.854gm
Valine	3.512 gm
Arginine	4.147 gm

Histidine	1.085 gm
Alanine	4.515 gm
Aspartic acid	5.793 gm
Glutamic acid	8.386 gm
Glycine	3.099 gm
Proline	2.382 gm
Serine	2.998 gm
Vitamins	**Quantity**
Thiamine (B1)	2.38 mg
Riboflavin (B2)	3.67 mg
Niacin (B3)	12.82 mg
Pantothanic acid (B5)	3.48 mg
Vitamine (B6)	0.364 mg
Folate (B9)	94 µg
Vitamine (B12)	0 µg
Choline	66 mg
Vitamine C	10.1 mg
Vitamine D	0 IU
Vitamine E	5 mg
Vitamine K	25.5 µg

Minerals	**Quantity**
Calcium	120 mg
Iron	28.5 mg
Magnesium	195 mg
Manganese	1.9 mg
Phosphorus	118 mg

Potassium	13630mg
Sodium	1048 mg
Zinc	2 mg
Other constituent	**Quantity**
Water	4.68 gm

Ashwagandha:-

Ashwagandha is packed with essential nutrients, including important vitamins and minerals. Some of the nutrients present in this plant include vitamin C, Iron, and Calcium.

A 100-gram serving of ashwagandha consists of the following quantities of nutrients:

Energy: 245 Kcal

Carbohydrate: 49.9 gm

Dietary Fiber: 32.3 gm

Protein: 3.9 gm

Iron: 3.3 mg

Calcium: 23 mg

Vitamin C: 3.7 mg

Beetroot :-

Beets mainly consist of water (87%), carbs (8%), and fiber (2–3%).

One cup (136 grams) of boiled beetroot contains fewer than 60 calories, while ¾ cup (100 grams) of raw beets boasts the following nutrients (1Trusted Source):

Calories: 43

Water: 88%

Protein: 1.6 grams

Carbs: 9.6 grams

Sugar: 6.8 grams

Fiber: 2.8 grams

Fat: 0.2 grams

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Apricot:-

One raw apricot (35g) provides 17 calories, 0.5g of protein, 3.9g of carbohydrates, and 0.1g of fat. Apricots are a good source of potassium, vitamin A, and phosphorus. The following nutrition information is provided by the USDA.1

Apricots, raw. FoodData Central. U.S. Department of Agriculture. [12]

Calories: 17

Fat: 0.1g

Sodium: 0.4mg

Carbohydrates: 3.9g

Fiber: 0.7g

Sugars: 3.2g

Protein: 0.5g

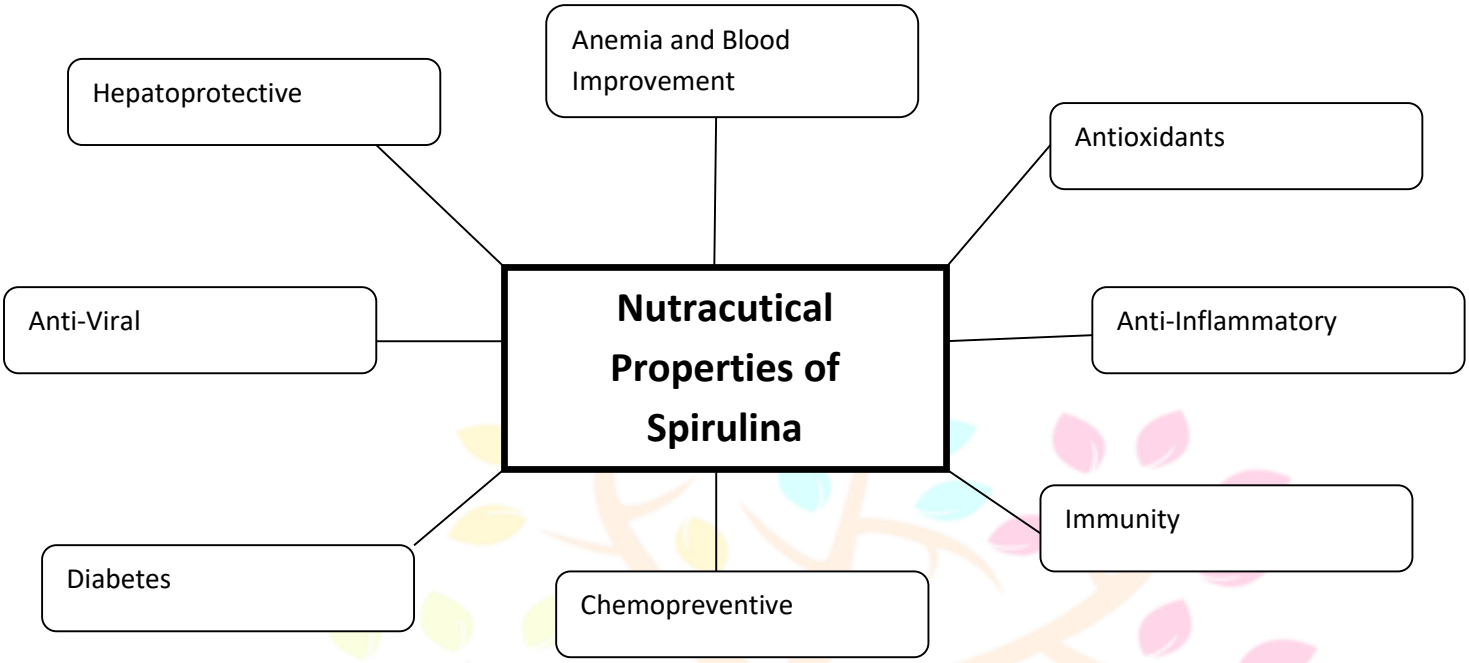
Potassium: 90.6mg

Vitamin A: 33.6mcg

Phosphorus: 8.1mg

Nutracutical Properties :-

- **Spirulina**



- **Ashwagandha:-**

Adaptogenic: Helps the body manage stress and anxiety.

Anti-inflammatory: May reduce inflammation in the body.

Cognitive support: Potential benefits for improving cognitive function and memory.

Energy boost: Believed to increase energy levels and combat fatigue.

Immune support: Thought to support immune system function.

Antioxidant: Contains compounds that may help protect against oxidative stress.

Sleep aid: Some studies suggest it may improve sleep quality.

Hormonal balance: Could help regulate hormones and support reproductive health.

Muscle strength: May enhance muscle strength and recovery. [13]

- **Beetroot**

Antioxidant: Contains betalains and other compounds that help fight oxidative stress and reduce inflammation.

Blood pressure regulation: High in nitrates, which may help lower blood pressure by relaxing blood vessels.

Exercise performance: Consumption of beetroot juice has been linked to improved endurance and exercise performance due to its nitrate content.

Brain health: Beetroot may support cognitive function and improve blood flow to the brain, potentially reducing the risk of dementia.

Digestive health: Rich in dietary fiber, which aids digestion and promotes gut health.

Detoxification: Compounds in beetroot support liver function and help the body eliminate toxins.

Anti-inflammatory: Betalains have anti-inflammatory properties that may help reduce inflammation in the body.

Heart health: Beetroot consumption may lower the risk of heart disease by improving cholesterol levels and reducing oxidative stress.

Nutrient-rich: Contains vitamins, minerals, and phytonutrients like vitamin C, folate, potassium, and manganese. [15,16,17]

- **Apricot**

Antioxidants: Apricots are packed with antioxidants like vitamin C and beta-carotene, which help neutralize harmful free radicals in the body, supporting immune function and reducing the risk of chronic diseases.

Fiber: They are a good source of dietary fiber, which aids digestion, promotes regular bowel movements, and helps maintain a healthy weight by increasing satiety.

Potassium: Apricots contain potassium, an essential mineral that helps regulate blood pressure, support muscle function, and maintain electrolyte balance.

Heart Health: The combination of antioxidants and potassium in apricots may contribute to heart health by reducing the risk of cardiovascular disease and promoting healthy cholesterol levels.

Anti-inflammatory: Some studies suggest that certain compounds found in apricots may have anti-inflammatory properties, which could help reduce inflammation in the body and alleviate symptoms of inflammatory conditions.

Potential anti-cancer properties: Preliminary research indicates that apricots may contain compounds with anti-cancer properties, although more studies are needed to fully understand their effects on cancer prevention and treatment.[14]

MATERIALS USED

Organic spirulina was purchased from neuherbs, Global Healthfit Retail (INDIA), Apricot was purchased from Nutri Organics farms Pune , Geletin capsule shell was purchases from Bioagri lifecare International premium size 0 (500 mg), Organic Beetroot was purchased from market and powder prepared in laboratory. Ashwagandha was purchased from herbs and powder prepare in laboratory ,Instrument were used for characterization are Electronic balance, FTIR (Shimadzu

Corporation), UV-Vis spectrophotometer (Thermo scientific) and Dissolution tester (Bezif company) etc.

PHYTOCHEMICAL STUDIES

Test of Protein in Spirulina was performed using Biuret test and Ninhydrine test.

METHODS OF FILLING

All Composition & Ingredients, Table 1, was weigh with starch as per their required quantity Respectively. Added one by one ingredient in starch base with gentle mixing. After mixing passed the mixture through mesh no. 180

Then ingredients were to be mixed using trituration to a fine and uniform powder. Then the Mixed powder was transferred on paper and flattened with a spatula in such that the layer of the powder is not greater than about 1/3 of the length of the capsule which was being filled. The cap was removed from the selected capsules and held in the left hand; the base was pressed repeatedly against the powder until it was filled. The spatula helps in filling the Capsule by pushing the last quantity of the materials into the base.

Table 1: Composition and Ingredients used for formulation

Ingredients	Each Capsule (500 mg) Contains	For 300 capsule . Batch
Organic Spirulina Powder	150 mg	45 gm
Ashwagandha Powder	50 mg	15 gm
Beetroot Powder	50 mg	15 gm
Apricot Seed Powder	50 mg	15 gm
Starch as Excipients	200 mg	60 gm

EVALUATION OF PREPARED FORMULATION

WEIGHT VARIATION TEST

First of all randomly 20 filled capsules was selected. Weighted the all 20 capsules

Collectively, and find out average weight by applying this formula.

$$\text{Average weight} = \frac{\text{Wt. of 20 Capsules}}{20}$$

Then weighted each 20 capsules one by one and note down their respective weights then Find Out percentage weight variation for each capsule with using formula.

$$\%\text{Weight Variation} = \frac{\text{Real Wt.- Avg. Wt. \% Weight Variation X100}}{\text{Avg. Wt}}$$

Maximum positive to maximum negative range was selected.

DISSOLUTION STUDIES

The dissolution test was performed for capsule using usp dissolution apparatus in which capsules basket type dissolution apparatus was used. The 900 ml of the 1.3 ph hydrochloric buffer as dissolution medium was introduced into the vessel of the apparatus. The dissolution medium was warmed to 36.50-37.50c. For this apparatus was operated immediately at the speed of 50 rpm for two hrs. After each 15 min a 10 ml specimen was withdrawn from a zone midway between the surface of the dissolution medium and top of the rotating blade or basket, in evaporating dish. Filter the specimen. For the each of the capsule tested, the amount of dissolved active ingredient in the solution was calculated as a percentage dissolved in 1.5 hrs. [18-19]

STABILITY STUDY

The stability of optimized formulation was tested according to International conference on Harmonization Guidelines an also per WHO Guideline. The formulation was stored at Accelerated (40±2ºC/ 75% ± 5%RH) condition wrapped in aluminum foil and kept in Humidity chamber. The stability study were conduct after 30, 60, 90 days. Similarly Formulation study at 30, 40, 50 ºC as per WHO Guideline. At the end of three month capsule Were tested for physical appearance, Disintegration Time, Drug Content, Drug Release and Self-life of drug was calculated.[20-21]

CONTENT UNIFORMITY

The procedures described by [22,23,24] were modified and used. A dose (one capsule) was emptied into a beaker and dissolved in 100 mL of 0.1 M HCL. It was then filtered into a 100 mL volumetric flask and topped up with 0.1 M HCL to the 100 mL mark. A volume of 10 mL was drawn from the prepared solution and transferred into a different volumetric flask. It was topped up to the 100 mL mark with 0.1M HCL. The absorbance of the solution from each product was determined using a UV-Vis spectrophotometer at the maximum wavelength previously recorded. The procedure was repeated for nine other capsules from each formulated product.

DISINTEGRATION TEST FOR FORMULATED CAPSULE

The procedure in [24] was used. The bath was filled with water to the desired mark and the temperature was set at 37°C ± 0.5°C. The beaker was filled with 600 mL of distilled water and suspended in the main bath. The temperature was allowed to reach equilibrium with that of the bath. One capsule was put into each of the six tubes. A disc was placed on each capsule to prevent it from floating. A watch clock was set and the apparatus was operated until all six capsules had disintegrated leaving only remnants of gelatin shell on the mesh. The procedure was repeated twice for each formulation.

RESULT AND DISCUSSION

Result of prefomulation study organoleptic studies is reported in table 2.M. P was found for spirulina, Ashwagandha, Beetroot and Apricot is 235-237°c, 188-190°c ,196-198°c and -10 to 18°c respectively. Soluble in methanol, 0.1 M HCl , ethanol and propylene glycol. Micrometric results indicate good flow properties reported in table 3. FTIR spectra of spirulina Ashwagandha, Beetroot and Apricot are reported in Fig. no 5, 6 7, 8 respectively. Calibration curve was reported in Fig.9, 10, 11, 12 . Phytochemical studies of spirulina reported in table 4 . Weight variation range was found to be -0.06% to +0.92% , Table 5.

Table 2: Results of organoleptic studies

Sr.No	Parameter	Spirulina	Ashwagandha	Beetroot	Apricot
1.	**Colour**	**Green**	**Light brown**	**Bright red**	**Tan and Brown**
2.	**Odour**	**Characteristic**	**Characteristic**	**Earthy smell**	**Neutral**
3.	**Taste**	**Bitter**	**Bitter**	**Slightly Sweet**	
4.	**Appearance**	**Powder**	**Powder**	**Powder**	**Powder**

Table 3: Results of micrometric properties

Drug	Angle of repose	Bulk Density	Tapped Density
Spirulina	**28.5 °**	**0.6 gm/ml**	**0.75gm/ml**
Ashwagandha	**27.80°**	**0.41gm/ml**	**0.55gm/ml**
Beetroot	**26°**	**0.55gm/ml**	**0.71gm/ml**
Apricot	**28.2°**	**0.64gm/ml**	**0.76gm/ml**

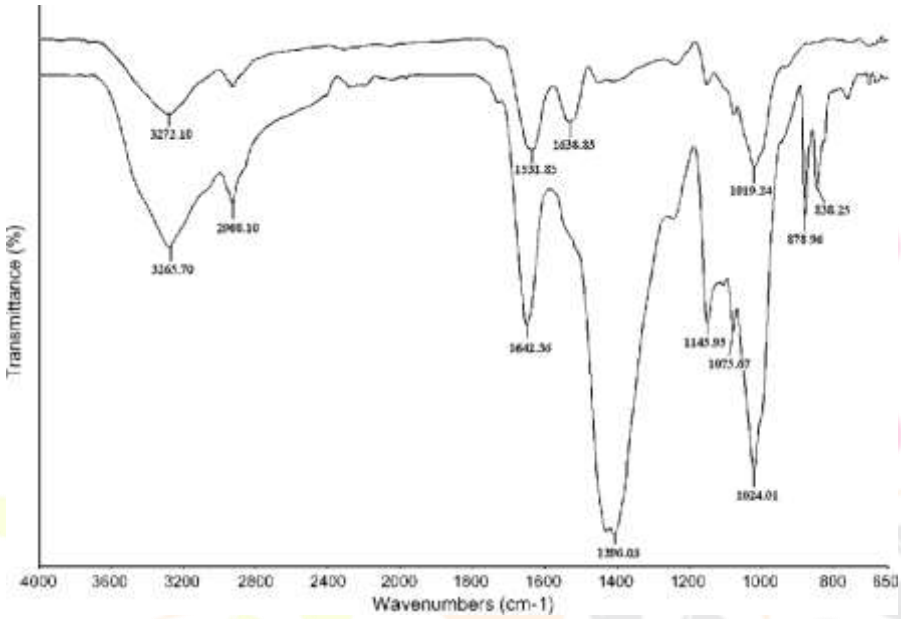


Fig 5. FTIR Spectra of Spirulina

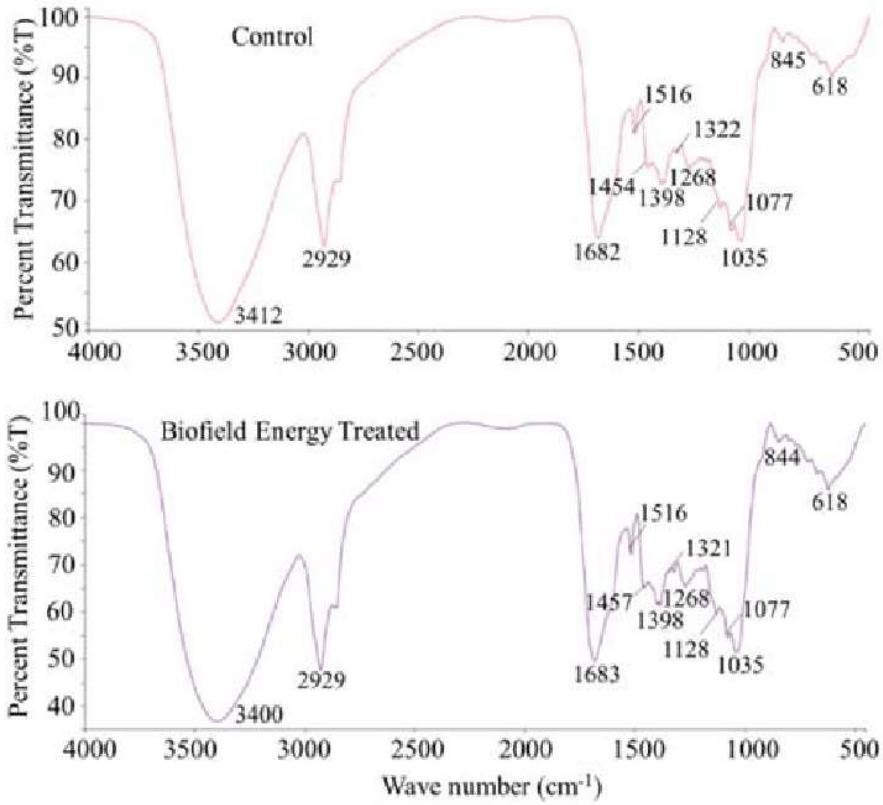


Fig.6. FTIR Spectra of Ashwgandha

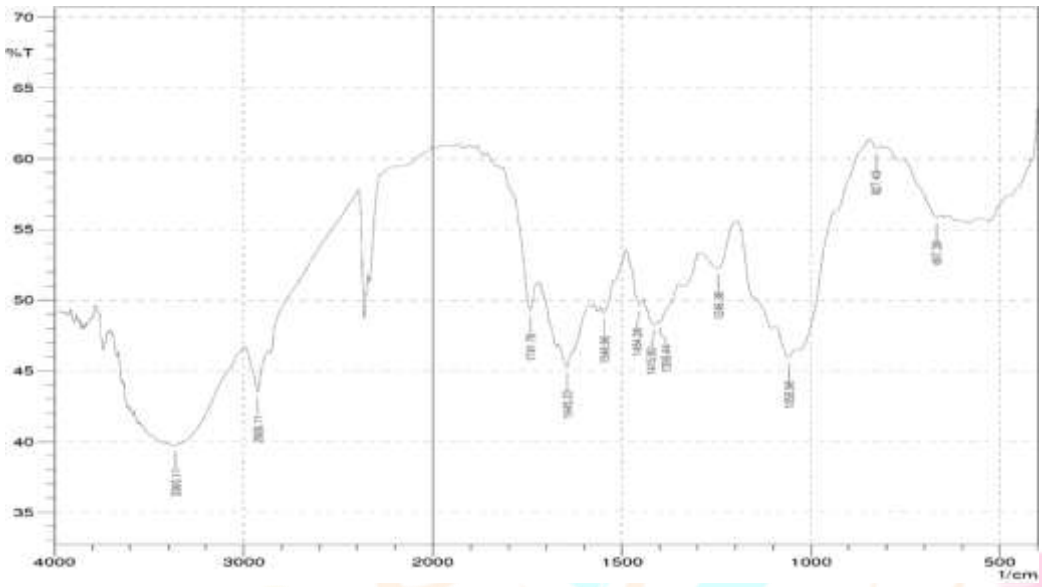


Fig 7.. FTIR Spectra of Beetroot

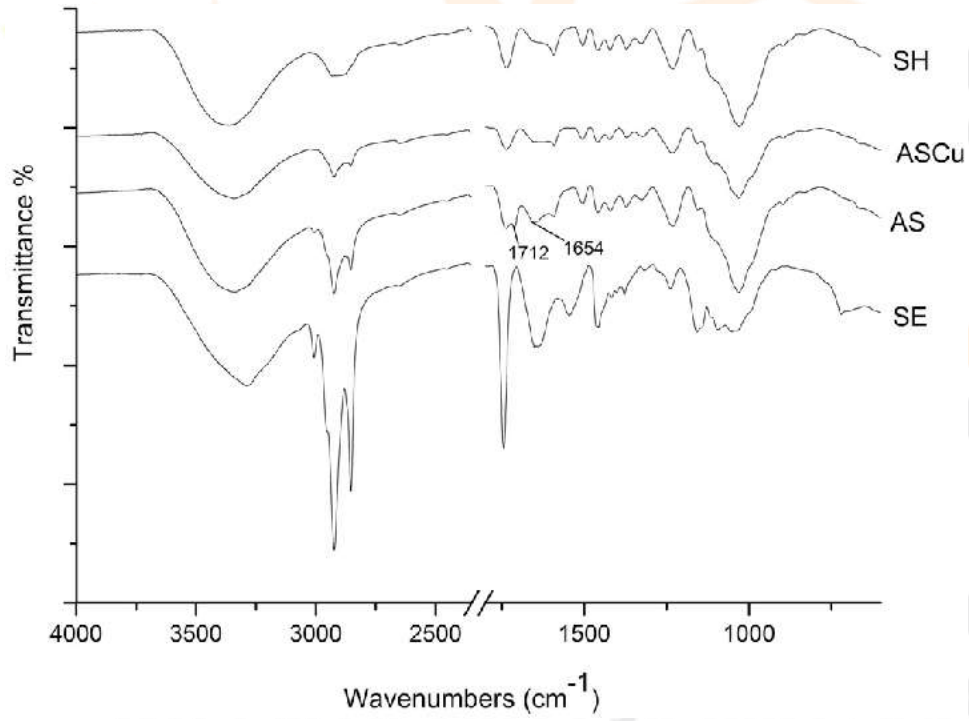


Fig 8. FTIR Spectra of Apicot

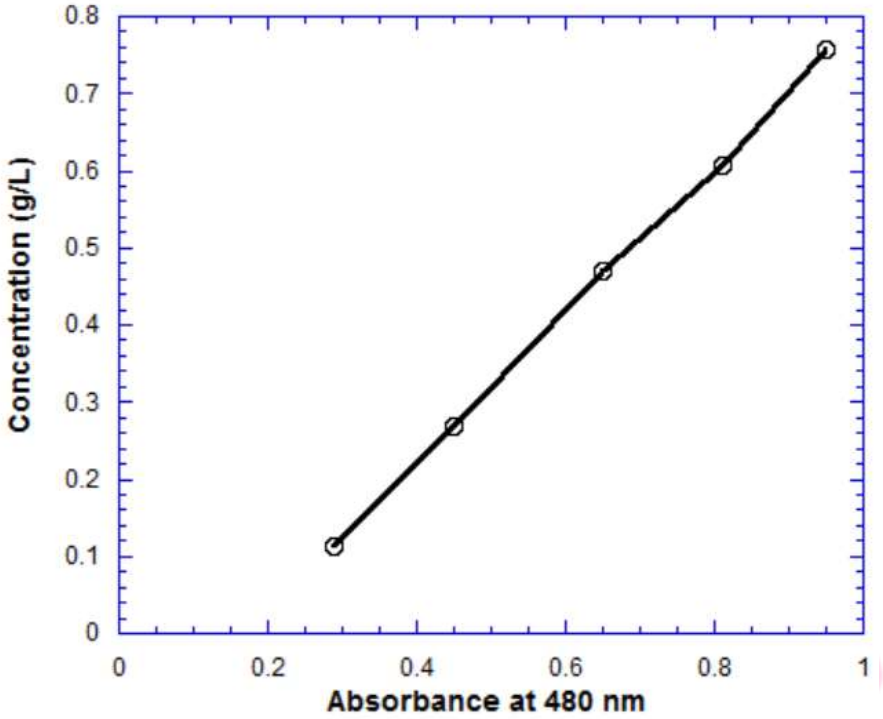


Fig .9. Calibration curve of Spirulina

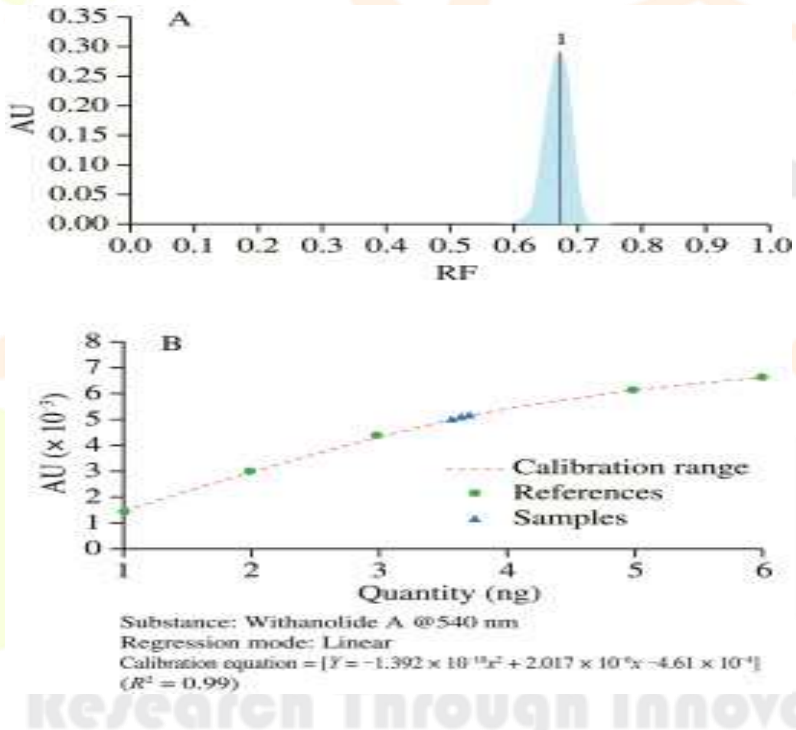


Fig 10. Calibration curve of Ashwgandha

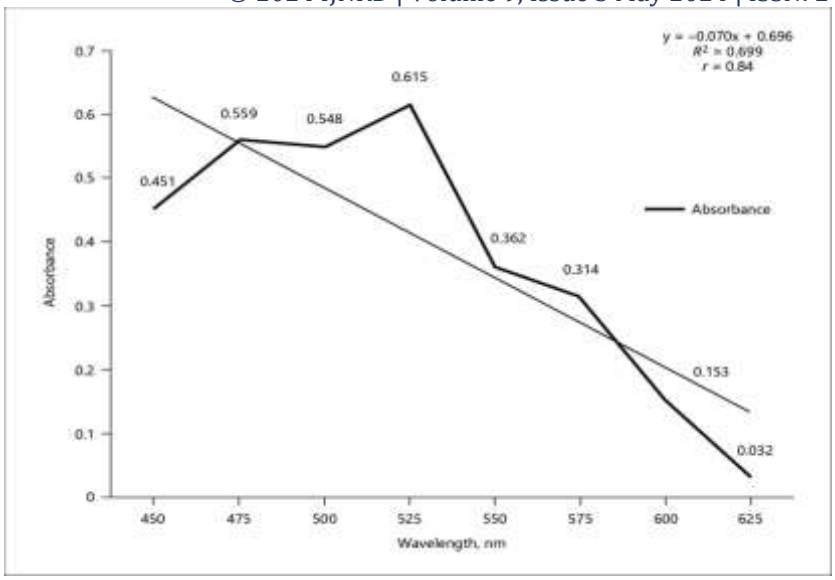


Fig 11. Calibration curve of Beetroot

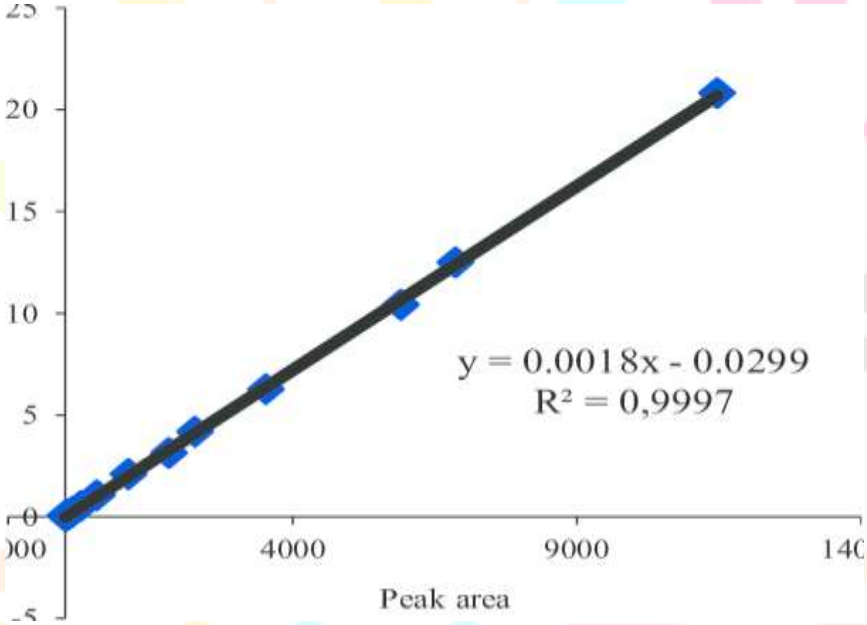


Fig .12. Calibration curve of Apricot

Table 4. : Phytochemical Studies of Spirulina

Test	Observation	Result
Biuret Test	Gives violet color	Presence of amino acids
Ninhydrine Test	Gives purple color	Presence of Proteins

Table 5 . : Results of weight variation

No.	Weight(In mg)	%Wt. variation	No.	Weight(In mg)	%Wt. variation
1	605	+0.09%	11	608	+0.59%
2	602	-0.39 %	12	603	-0.23%
3	608	+0.59%	13	599	-0.89%
4	606	+0.26%	14	609	+0.76%
5	603	-0.23%	15	606	+0.26%
6	600	-0.72%	16	605	+0.09%
7	601	-0.56%	17	604	-0.06%
8	604	-0.06%	18	601	-0.56%
9	609	+0.76%	19	603	-0.23%
10	610	+0.92%	20	602	-0.39 %

Table .6 Result of Dissolution Study

Time (min)	Abs.of Capsule	Conce. Of Capsule In 900 ml	% of Capsule release
0	0.0000	00	00
15	0.0075	18	10.5
30	0.0088	22	11.5
45	0.0101	86	43.00
60	0.0120	152	74.00
75	0.0123	154	77.00
90	0.0131	189	92.00

Table no.7 : Result of Stability Studies

S.No	Parameter		Day 0	Day 30	Day 60	Day 90
1	% weight Variation		-0.06% to	-0.09%	-0.05% to +0.92%	+0.26%
2	Disintegration Time		4 min . 10 sec	4 min . 45 sec	5 min . 10 sec	6 min 43 sec
3	Drug Content	Drug A	198 mg	195 mg	189 mg	185 mg
		Drug B	99	97.50	95.85	93.50
		Drug C	99	96.5	93.5	92.3
		Drug D	99	98.4	96.6	94.6
4	% Drug Release	Drug A	99	97.3	96.8	95.4
		Drug B	99	98.4	96.2	94.6

		Drug C	99	96.4	94.9	92.7
		Drug D	99	97.4	95.1	93.8

CONCLUSIONS

The formulation and evaluation of a health immunity booster capsule, integrating Spirulina with Ashwagandha, Beetroot, and Apricot, has yielded encouraging outcomes. This synergistic blend holds promise for bolstering the immune system. Spirulina, a nutrient-rich algae, is renowned for its immune-boosting properties due to its high content of vitamins, minerals, and antioxidants. Ashwagandha, an adaptogenic herb, has been traditionally used to enhance immunity and combat stress. Beetroot, abundant in vitamins and minerals, possesses immune-boosting and anti-inflammatory properties. Apricot, rich in vitamin C and other antioxidants, contributes further to immune support. The combination of these ingredients likely amplifies their individual benefits, potentially creating a more potent immune-boosting effect. Through rigorous evaluation, this formulation has demonstrated potential efficacy in enhancing immunity. Further research is warranted to optimize dosages, assess long-term effects, and validate its effectiveness in diverse populations. Additionally, investigating potential mechanisms of action and conducting clinical trials could provide deeper insights into the capsule's efficacy and safety profile. Overall, this formulation presents a promising avenue for developing natural, synergistic approaches to support immune health and overall well-being.

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