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A novel polyherbal syrup with antioxidant potential: formulation, physicochemical characterization, and prospects for stress management

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ABSTRACT

The present study was aimed to develop and formulate a polyherbal syrup from the roots of *Asparagus racemosus* and *Withania somnifera*, leaves of *Andrographis paniculata* and *Ocimum sanctum*, and fruits of *Piper nigrum* to mitigate the vaccine-induced stress in animals. The formulation was prepared using traditional decoction method, combined with a simple syrup base and pharmaceutical preservatives. The physicochemical evaluation of the syrup revealed a reddish-brown color, aromatic odour, sweet taste, pH of 6.7, density of 1.180 g/ml, and a viscosity suitable for an oral liquid dosage form. Stability studies on the preparation showed no significant changes in these parameters after one month of storage at 4 °C and room temperature. Qualitative phytochemical screening of the preparation confirmed the presence of alkaloids, flavonoids, phenolics, and tannins. Quantitatively, the syrup exhibited a high total phenolic (158.30 ± 3.38 mg GAE/g) and total flavonoid (123.70 ± 0.88 mg QE/g) content. The formulation demonstrated potent in vitro antioxidant activity with an IC₅₀ value of 86.21 µg/ml in the DPPH assay. The results indicate that the developed polyherbal syrup is a stable and pharmaceutically viable formulation with significant antioxidant potentials and these findings support its potential use as a supplement for alleviating vaccine induced stress and further warranting in-vivo studies to evaluate its immunomodulatory efficacy in animals.

Keywords: Polyherbal Syrup, Phytochemistry, DPPH Assay, Anti-Stress, Immunomodulation

Introduction

Vaccination against diseases is an essential protocol for preventing diseases in animals, but often induces a transient response characterized by oxidative stress and reduced productivity, which primarily impacts the farmer's livelihood. Hence, addressing this vaccine stress with safe, cost-effective, immunomodulators is of high practical importance. Herbal remedies offer a viable solution rooted in traditional knowledge. This study focuses on developing a polyherbal syrup combining five well-known Indian medicinal plants *Asparagus racemosus*, *Withania somnifera*, *Andrographis paniculata*, *Ocimum sanctum* and *Piper nigrum* selected for their synergistic, adaptogenic and immunostimulant properties. The aim was to create a stable, palatable, and easy-to-administer formulation to support goat's health during the critical post-vaccination period. This paper reports the development of polyherbal formulation, physicochemical evaluation and *in-vitro* antioxidant potential.

Materials and Methods

Selection, collection and authentication of medicinal plants

The roots of *Asparagus racemosus* and *Withania somnifera*, leaves of *Andrographis paniculata* and *Ocimum sanctum* and fruits of *Piper nigrum* were selected based on traditional Indian medicinal use and scientific support. Plant materials were sourced from the Ethno-Veterinary herbal garden, Veterinary College and research Institute, Orathanadu, Thanjavur, Tamil Nadu. Botanical authentication was done by the Siddha Central Research Institute, Chennai, Tamil Nadu, the institute functions under the Central Council for Research in Siddha, Ministry of AYUSH, Government of India. The authenticated herbarium specimens were deposited at EVHPR&D Centre, VCRI, Orathanadu (Voucher No. 19 -23/EVHPR&D Centre/2023).

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Development of polyherbal formulation

Method of preparation of decoction

The oral polyherbal formulation was crafted using the aqueous decoction method of extraction (Shailaja *et al* (Shakeel *et al.*, 2015) [1]). To initiate this process, powdered raw materials of the five selected plants were combined in equal proportions. The decoction method requires the addition of water at a ratio of 16 times the weight of the raw powder. The mixture was then heated until it boiled and subsequently condensed to approximately one-fourth of its initial volume. After boiling, the mixture was allowed to cool and subsequently filtered. The resulting filtrate was left to stand overnight and was subsequently used in the preparation of the final polyherbal syrup.

Method of preparation of simple syrup

The simple syrup was prepared by blending 66.7 % sugar with water and was heated until the sugar dissolved completely, with continuous stirring.

Preparation of polyherbal syrup

The polyherbal syrup was meticulously formulated by combining the decoction and the simple syrup in a precise ratio of 1:5 (Thangarathinam *et al* (Yahfoufi *et al.*, 2018) [2]). Additionally, specific quantities of methyl paraben sodium, propyl paraben sodium, sodium benzoate, bronopol, citric acid, saccharin sodium and a mixed fruit flavoring agent were added to the mixture (Rowe *et al* (Shankar *et al.*, 2016) [3]) and the exact composition (in %) of these ingredients for the polyherbal formulation are listed in table 1.

Evaluation of the polyherbal formulation

The polyherbal formulation underwent a comprehensive assessment of its physicochemical properties (Karishma *et al* (Madaan *et al.*, 2011) [4], Senthilkumar *et al* [5], Shakeel *et al* (Thangarathinam *et al.*, 2013) [6]). The parameters include colour, odour, taste and pH. To determine pH, precisely measured 10 ml of the final syrup was transferred to a 100 ml volumetric flask and diluted with distilled water to reach a total volume of 100 ml. The solution underwent sonication for approximately 10 minutes, after which the pH was measured using a digital pH meter.

Density and specific gravity

The determination of density and specific gravity of the syrup and its vital physicochemical properties were carried out using a well-defined procedure (Senthilkumar *et al* [5]) to ensure precision and accuracy. The specific gravity bottle was meticulously cleaned with chromic acid. The bottle was rinsed at least two to three times with distilled water. The weight of the empty dry bottle with a capillary tube stopper (w_1) was measured. The bottle was filled with the water and sealed with the stopper. Excess liquid on the outside of the tube was gently wiped off using tissue paper. The bottle with the unknown liquid was weighed on an analytical balance (w_2). The weight in grams of the unknown liquid (w_3) was calculated. The syrup's density was calculated using the formula:

$$\frac{w_3 - w_1}{w_2 - w_1} \times \text{Density of water}$$

Specific gravity was calculated using the formula: $\frac{w_3}{w_2}$

Viscosity

Viscosity measurements were carried out using a Brookfield viscometer (Shankar *et al* (SenthilKumar *et al.*, 2022) [5]) with an S18 spindle. Viscosity was assessed at varying shear rates, including 200 rpm, 150 rpm, 100 rpm, 60 rpm and 30 rpm.

Stability testing

The stability of the prepared polyherbal formulation was determined by exposing ten portions of the final syrup (A1, A2, B1, B2, C1, C2, D1, D2, E1 and E2) to accelerated temperature conditions. These

samples were stored in amber-colored glass bottles at a temperature of 4°C and room temperature. At intervals of 24 hours, 48 hours, 72 hours and 1 month the samples were evaluated for the physicochemical parameters, turbidity and homogeneity with the objective of detecting any alterations in their properties.

Qualitative phytochemical screening

The extracts of the selected plants and the PHF were subjected to phytochemical screening to know the presence of secondary metabolites which includes alkaloids, saponins, phenolic compounds, tannins, sterols, triterpenoids, flavonoids, proteins, amino acids, carbohydrates and glycosides (Shaikh and Patil (Shailaja *et al.*, 2017) [7]). Procedures for the different phytochemical screening were mentioned in table 2.

Quantitative analysis of polyherbal formulation

In the quantitative analysis of the PHF, the determination of both total phenolic content (TPC) and total flavonoid content (TFC) was conducted by Folin-Ciocalteu method and Aluminum Chloride Colorimetric Assay, respectively (Madaan *et al* (Majumdar *et al.*, 2021) [8], Phuyal *et al* (Rajmane *et al.*, 2020) [9]).

Estimation of total phenolic content (TPC)

The concentration of phenolic compounds in the PHF was determined using the Folin-Ciocalteu method. Standard gallic acid solution and the PHF were prepared by dissolving them in methanol. Various concentrations of both the gallic acid solution and the PHF (6.25, 12.5, 25, 50, 75, and 100 µg/ml) were prepared.

One ml of aliquot of each concentration was diluted with 10 ml of distilled water and added with 1.5 ml of Folin-Ciocalteu reagent (FCR) and then incubated at room temperature for 5 minutes, then 4 ml of 20% Na₂CO₃ was added and the volume was adjusted with distilled water to 25 ml. The resulting blue-coloured mixture was thoroughly mixed and incubated for 30 minutes at room temperature. After incubation, the absorbance was measured at 765 nm against a blank. The FCR reagent oxidizes phenolic compounds in the PHF, causing it to change into a dark blue color, which was then quantified using a UV-visible spectrophotometer. The total phenolic content of the PHF was expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample in dry weight (mg/g).

Estimation of total flavonoid content (TFC)

The total flavonoid content in the PHF was determined through an Aluminum Chloride Colorimetric Assay. Stock solutions (4 mg/ml) of quercetin and the PHF were prepared by dissolving them in methanol. Then, both the standard solution and the sample were subsequently serially diluted to create various concentrations of 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml and 1 mg/ml solutions. For each concentration, 1 ml of quercetin and 1 ml of the PHF were added in a test tube containing 4 ml of distilled water. Simultaneously, 0.3 ml of 5% NaNO₂ was introduced into the test tube, followed by an addition of 0.3 ml of 10% AlCl₃ after 5 minutes. Subsequently, 2 ml of 1 M NaOH was added to the mixture after 6 minutes. The volume of the mixture was adjusted to 10 ml by promptly adding 4.4 ml of distilled water. The flavonoid content was expressed as quercetin equivalent (mg QE/g) using a linear equation derived from the standard calibration curve.

Determination of in vitro antioxidant activity

2, 2 diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity

The free radical scavenging capacity of the PHF was assessed using the DPPH assay (Harichandan *et al* (Hossain *et al.*, 2012) [10]). The DPPH solution (0.004% w/v) was prepared by dilution in methanol. Stock solutions of the polyherbal formulation (1 mg/ml) and standard ascorbic acid (0.05 g/ml) were prepared using methanol. Various concentrations (10-500 µg/mL) of the polyherbal formulation and ascorbic acid were placed in test tubes. To each test tube, 1 ml of freshly prepared DPPH solution was added and the test tubes were shielded

from light by covering them with aluminum foil. The final volume in each test tube was adjusted to 2 ml with methanol and the mixtures were incubated in the dark for 30 minutes at room temperature. After incubation, the absorbance was measured at 517 nm using a spectrophotometer. A control sample was prepared, which contains the same volume of methanol and DPPH without any polyherbal formulation, and another one with reference ascorbic acid. Methanol was used as the blank. The calculation of free radical scavenging was performed using the following formula:

Results

Evaluation of polyherbal formulation

Physicochemical properties

The polyherbal formulation showed acceptable physicochemical properties. It had a reddish-brown colour, aromatic odour and sweet taste, including good palatability. The pH (6.7) was near neutral, suitable for oral use. The density (1.180 g/ml) and specific gravity (1.184) confirmed proper consistency. The viscosity were found to be 13.0 cP, 12.9 cP, 12.7 cP, 12.6 cP, 11.7 cP at 200 rpm, 150 rpm, 100 rpm, 60 rpm, 30 rpm, respectively, indicating good flow properties. The stability testing showed that there was no characteristic changes in PHF, stored after one month and the results were showed in table 3.

Qualitative phytochemical screening

The results of the qualitative phytochemical screening of the individual extracts and the PHF are summarized in table 4. The individual extracts and the PHF showed the presence of carbohydrates, proteins, amino acids, alkaloids, glycosides, saponin glycosides, flavonoids, phenols, tannins, sterols and terpenoids. The carbohydrate is absent in *Andrographis paniculata* and *Ocimum sanctum*. In *Asparagus racemosus*, proteins and amino acids were absent. Cardiac glycosides is absent in all the selected plants, whereas the PHF exhibited the presence of all phytochemical constituents, except cardiac glycosides.

Quantitative analysis of polyherbal formulation

Total phenolic content (TPC) and total flavonoid content (TFC)

The total phenolic content of individual herbs and PHF were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (GAE) and is showed in table 5 and the total flavonoid content was expressed in terms of quercetin equivalent and the results were shown in table 6.

Quantitative analysis of polyherbal formulation

Total phenolic content (TPC)

Phenolic compounds are a diverse group of secondary metabolites found in plants and are known for their antioxidant properties. A higher total phenolic content is generally associated with increased antioxidant activity, which can be beneficial by neutralizing free radi-

In vitro antioxidant activity

2, 2 diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity

The DPPH free radical scavenging activity exerted by PHF as well as ascorbic acid were summarized in table 7 and figure 1. The IC₅₀ of PHF was found to be 86.21 µg/ml.

Discussion

Evaluation of polyherbal formulation

In the present study, the PHF exhibited a reddish brown color, sweet taste and neutral pH. The pH obtained from the prepared polyherbal formulation was 6.7, which is correlated with Rajmane *et al* (Rowe *et al.*, 2006) [11], reported the pH of antitussive syrup was 6.98 and 6.51. Maintaining a near-neutral pH is essential as it enhances formulation stability, minimizes irritation and ensures compatibility with biological systems. The density of the PHF was 1.18 g/ml, which is close to 1.43 g/ml of anti-tussive syrup prepared by Rajmane *et al* (Rowe *et al.*, 2006) [11] and almost nearer to 1.06 g/ml of a polyherbal syrup prepared by Patil *et al* (Phuyal *et al.*, 2020) [12]. This indicates uniformity in composition and proper solubilization of ingredients in preparation. The specific gravity of the PHF was found to be 1.184 which correlates to the findings of Karishma *et al* (Madaan *et al.*, 2011) [4] and Thangarathinam *et al* (Yahfoufi *et al.*, 2018) [2], reported as 1.26 and 1.18, respectively for polyherbal syrups. It ensures the consistency and reproducibility of the formulation. The viscosity of the PHF was 13 cP, which is similar to the findings of 17.05 cP viscosity of polyherbal syrup by Karishma *et al* (Madaan *et al.*, 2011) [4]. This moderate viscosity ensures ease of administration while maintaining adequate suspension of active constituents, thereby preventing rapid sedimentation. Stability ensures a drug product meets defined standards for identity, strength, purity, and quality over a specific storage period. All components, whether active molecules or excipients, can impact stability. Additionally, particle size, pH, container properties, and the presence of contaminants affect the stability of the drug product. The goal of the present investigation is to maintain these essential characteristics within predefined limits. The prepared PHF were stored for a period of one month and does not show any changes in the physicochemical characters.

Qualitative phytochemical screening

The phytochemical screening of individual extracts and the PHF revealed the presence of flavonoids, alkaloids, glycosides, phenolic compounds, tannins, terpenoids, sterols, carbohydrates, proteins and amino acids. The immunomodulatory properties are mainly due to the presence of phenols and flavonoids. Flavonoids and phenolic compounds contributed antioxidant and anti-inflammatory effects (Cecchini *et al* [13]), alkaloids might have interacted with immune cells, glycosides influence cellular signaling, tannins affect cytokine production, and terpenoids and sterols possessed antimicrobial properties. The present study's findings, especially those related to the identified phytocomponents are in agreement with the results reported by Majumdar *et al* (Patil *et al.*, 2019) [14], who demonstrated the immunomodulatory, antioxidant and diverse pharmacological activities of the polyherbal formulation.

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

cals and reducing oxidative stress (Yahfoufi *et al* (Zahiruddin *et al.*, 2022) [15]). In this study, the polyherbal formulation showed the higher phenolic content which could be (158.30 ± 3.38) due to the synergistic action of the selected plants that might have acted as antioxidants and anti-inflammatory agents, supporting immune modulation by regulating responses and interacting with immune cells and signaling pathways. These results are in accordance with the findings of (Cecchini *et al*) [13], the higher phenolic and flavonoid content ob

served in the polyherbal formulation suggested their potential role in facilitating antioxidant immunomodulatory effects.

Total flavonoid content (TFC)

Flavonoids are a diverse group of polyphenolic compounds found in plants. The determination of total flavonoid content has been studied for their antioxidant, *anti*-inflammatory, antiviral, anticancer including immunomodulatory properties in various studies (Hosseinzade *et al* (Karishma *et al.*, 2021) [16]). In this investigation, the polyherbal formulation demonstrated an elevated flavonoid content, attributed to the synergistic effects of the chosen plants. Flavonoids showcase antioxidant properties by neutralizing free radicals, binding metal ions (such as iron and copper), and hindering enzymes responsible for free radical generation (Hossain *et al* (Hosseinzade *et al.*, 2019) [17]).

In-vitro antioxidant activity

2, 2 diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity

The IC₅₀ value of the polyherbal formulation was found to be 86.21 µg/ml which is less compared to the standard ascorbic acid (55.37 ± 1.25 µg/ml), revealed high *invitro* antioxidant property and almost similar to Zahiruddin *et al* [18] who showed the IC₅₀ (82.54 ± 2.54 µg/ml) of the prepared immunomodulatory polyherbal formulation comprises *Phyllanthus emblica* L., *Piper nigrum* L., *Withania somnifera* (L.) Dunal, and *Tinospora cordifolia* (Wild). The combined extracts (PHF) revealed a superior antioxidant potential compared to the individual ones. The IC₅₀ value denotes the concentration of substrate causing a 50% reduction in free radical activity. A lower IC₅₀ value indicates increased antioxidant activity in the combination. The plant extracts present in the polyherbal blend contained elevated levels of phenols and flavonoids, likely contributing to the observed antioxidant effects (Deepak and Anurekha (Harichandan *et al.*, 2019) [19]).

Table 1. Percentage composition of excipients used in polyherbal formulation

Name of Ingredients	Composition (%)
Methyl paraben sodium	0.10
Propyl paraben sodium	0.01
Sodium benzoate	0.20
Bronopol	0.01
Citric acid	0.10
Saccharin sodium	0.03
Mixed fruit flavor	0.10

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Table 2. Qualitative phytochemical screening of PHF

S. No.	Procedures	Inference	Result
1.	Test for alkaloids Mayer's test To the filtrate few drops of Mayer's reagent was added Dragendroff's reagent To the filtrate few drops of Dragendroff's reagent was added Hager's test To the filtrate few drops of Hager's reagent was added Wagner's test To the filtrate add few drops of Wagner's reagent	Cream colour precipitate Orange brown precipitate Yellow colour precipitate Reddish brown precipitate	Presence of alkaloids
2.	Test for saponins Foam Froth test To a small quantity of the extract 5 ml of distilled water was added and shake for 5 minutes in test tube	Foam remains for 2 minutes	Presence of saponins
3.	Test for phenolic compounds Add 3 drops of 10% ferric chloride to a small quantity of extract in a test tube	Blue or green colour appears	Presence of phenolic compounds
4.	Test for tannins To the extract, 1% of gelatin and 10% sodium chloride were added	White precipitate formed	Presence of tannins
5.	Test for sterols Liebermann – Buchard test To a small quantity of extract add 2 ml of acetic anhydride and add 2 ml of concentrated sulphuric acid along the sides of the test tubes	A brown colour ring at the junction of the two liquids	Presence of sterols
6.	Test for triterpenoids Salkowski test To the extract first add with chloroform and then add with few drops of concentrated Sulphuric acid, shaken and allowed standing for some time	Yellow colour appears at lower layer	Presence of triterpenoids
7.	Test for flavonoids Alkaline reagent test To the extract few drops of 1% sodium hydroxide solution was added To this colour, add 1 ml of dilute hydrochloric acid along the sides of the test tube Shinoda test To the extract, few fragments of magnesium ribbon was added followed by concentrated hydrochloric acid drop wise	Intense yellow colour appears Yellow colour disappears Appearance of Pink scarlet, Crimson red or green to blue colour	Presence of flavonoids
8.	Test for aminoacid and protein Ninhydrin test To 1 ml of extract add 1 ml of ninhydrin solution and heat at 50°C for 1 minute	Violet colour appears	Presence of aminoacid and protein
9.	Test for carbohydrates Molisch's test To the 0.5 ml of extract, add few drops of alcoholic alpha naphthol and 0.2 ml of concentrated sulfuric acid slowly through the sides of the test tube Benedict's test To a small quantity of extract add 2 ml of Benedict's reagent and heat gently	Purple to violet colour ring appears at the junction Reddish brown precipitate	Presence of carbohydrate
10.	Test for glycosides Legal's test To the extract add 2 drops of pyridine and alkaline sodium nitroprusside solution	Blood red colour appears	Presence of glycosides

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S. No.	Procedures	Inference	Result
11.	Test for cardiac glycosides Keller Killiani test To the extract add 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. It was transferred to a small test tube and then 0.5 ml of concentrated sulphuric acid was added carefully by the side of the test tube,	Blue colour appears in the acetic acid layer.	Presence of cardiac glycosides

Table 3. Stability Testing of PHF

Sample Code	Time Duration	Temperature	Physicochemical parameters						
			Colour	Odour	Taste	pH	Wt/ml at 25°C	Specific gravity	Turbidity/ Homogeneity
A1	0 hr	4°C	NC	NC	NC	6.7	1.18	1.184	NC
A2		Room temperature	NC	NC	NC	6.7	1.18	1.184	NC
B1	24 hrs	4°C	NC	NC	NC	6.7	1.18	1.184	NC
B2		Room temperature	NC	NC	NC	6.7	1.18	1.184	NC
C1	48 hrs	4°C	NC	NC	NC	6.7	1.18	1.184	NC
C2		Room temperature	NC	NC	NC	6.7	1.18	1.184	NC
D1	72 hrs	4°C	NC	NC	NC	6.7	1.18	1.184	NC
D2		Room temperature	NC	NC	NC	6.7	1.18	1.184	NC
E1	1 month	4°C	NC	NC	NC	6.3	1.18	1.186	NC
E2		Room temperature	NC	NC	NC	6.3	1.18	1.186	NC

Table 4. Qualitative Phytochemical Analysis of Individual Extract and Polyherbal Formulation

S. No	Test	<i>A. racemosus</i>	<i>W. somnifera</i>	<i>A. paniculata</i>	<i>O. sanctum</i>	<i>P. nigrum</i>	PHF
1		Carbohydrate					
	Molisch's test	Positive	Positive	Negative	Negative	Positive	Positive
	Benedict's test	Positive	Positive	Negative	Negative	Negative	Positive
2		Proteins and Amino acids					
	Millon's test	Negative	Positive	Positive	Positive	Positive	Positive
	Ninhydrin test	Negative	Negative	Negative	Negative	Negative	Negative
3		Alkaloids					
	Mayer's test	Positive	Positive	Positive	Positive	Positive	Positive
	Dragendroff's test	Positive	Positive	Negative	Negative	Negative	Positive
	Wagner's test	Negative	Positive	Positive	Positive	Positive	Positive
	Hager's test	Negative	Positive	Negative	Positive	Positive	Positive
4		Glycosides					
	Legal's test	Positive	Positive	Positive	Positive	Positive	Positive
5		Saponin glycosides					
	Foam froth test	Positive	Positive	Positive	Positive	Negative	Positive
6		Cardiac glycosides					
	Keller killiani test	Negative	Negative	Negative	Negative	Negative	Negative
7		Flavonoids					
	Shinoda test	Negative	Positive	Negative	Negative	Negative	Positive
	Alkaline reagent test	Positive	Positive	Positive	Positive	Positive	Positive
8		Phenolic compounds and tannins					
	Phenolic compounds	Positive	Positive	Positive	Positive	Positive	Positive
	Tannins	Positive	Negative	Positive	Positive	Positive	Positive
9		Sterols and Triterpenoids					
	Salkowski test	Positive	Positive	Negative	Negative	Positive	Positive

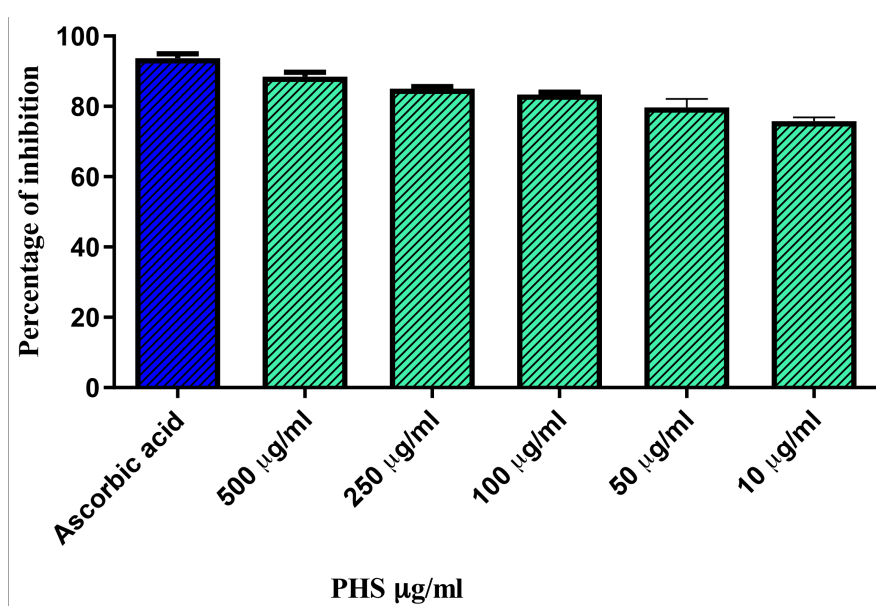
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Table 5. Total Phenolic Content of Individual Extracts and PHF

S. No	Plants	TPC (mg/g GAE)
1	<i>Asparagus racemosus</i>	87.42 ± 0.56
2	<i>Withania somnifera</i>	57.42 ± 1.13
3	<i>Andrographis paniculata</i>	68.16 ± 0.22
4	<i>Ocimum sanctum</i>	89.94 ± 0.19
5	<i>Piper nigrum</i>	97.19 ± 0.31
6	PHF	158.30 ± 3.38

Table 6. Total Flavonoid Content of Individual Extracts and PHF

S. No	Plants	TFC (mg/g QE)
1	<i>Asparagus racemosus</i>	152.70 ± 1.45
2	<i>Withania somnifera</i>	1.90 ± 0.06
3	<i>Andrographis paniculata</i>	1.10 ± 0.06
4	<i>Ocimum sanctum</i>	88.50 ± 0.29
5	<i>Piper nigrum</i>	98.00 ± 0.58
6	PHF	123.70 ± 0.88

**Figure 1.** DPPH Scavenging Activity of PHF - Percentage of Inhibition

Conclusion

The developed polyherbal formulation demonstrated good physico-chemical stability, significant antioxidant activity and the presence of key bioactive phytoconstituents. These findings suggest its potential

as a safe and effective formulation for mitigating vaccine induced stress and supporting immunomodulation in animals.

Table 7. DPPH Scavenging Activity of PHF - Percentage of Inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of Inhibition (in triplicates)			Mean Value (%)
1.	Ascorbic acid	95.12492	93.29677	92.68739	93.70303
2.	500 µg/ml	89.51859	88.78732	86.95917	88.42169
3.	250 µg/ml	85.55759	85.19196	84.39976	85.04977
4.	100 µg/ml	83.91225	83.60756	82.51066	83.34349
5.	50 µg/ml	82.44973	78.24497	78.48873	79.72781
6.	10 µg/ml	76.84339	75.86837	74.77148	75.82775

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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