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Research article

Phytochemical and preclinical evaluation of herbal formulation as a potent health supplement

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ABSTRACT

The present study was carried out with the objective to perform Quality control profiling of Polyherbal formulation in the form of capsules and to evaluate its potential for weight gain and cognitive functions. The formulation was the combination of different plants (Spirulina, Ashwagandha, Brahmi, Gokhru, Shankhpushpi, Mahua, Shingada, and Bael) that are traditionally used for anti-oxidant and immunomodulatory activity and also reported for a health supplement. The parts of different plants and roots were separately dried, powdered and then extracted to evaluate various physicochemical, phytochemical and pharmaceutical parameters. Preclinical evaluation of herbal formulation was done on Swiss Albino Mice which were randomly divided into 6 groups, with 6 in each group. First group served as control, Second group was treated with Standard Protein Powder the rest four groups had doses 5, 10mg/kg of body weight for short term and 15, 20mg/kg of body weight for long term respectively. The animals were kept under observation for a period of 21 days, after 21 days, the animals were sacrificed and vital organs were dissected, weighed and samples of the tissues were fixed for histological examination. The physicochemical and pharmaceutical parameters were within the prescribed limits per standard values mentioned in API. Phytochemical screening shows alkaloids, flavonoids, tannins, alkaloids; saponin and carbohydrates found to be in considerable amounts in polyherbal formulation and the total polyphenol was found to be absent. The HPLC analysis confirmed the presence of C-Phycocyanin in formulation, which was found to be 7.36% w/w. The polyherbal formulation of health supplement was found to significantly increase in the animals treated with the control, which was quite comparable to standard C-Phycocyanin.

Keywords: Polyherbal formulation, Physicochemical, Phytochemical, High-Performance Liquid Chromatography, C-Phycocyanin.

INTRODUCTION

In the developing world, malnutrition is still a significant public health issue, especially in southern Asia and sub-Saharan Africa.[1-5] Diets in populations are frequently deficient in macronutrients (protein, Carbohydrates and fat, leading to protein–energy malnutrition), micronutrients (electrolytes, minerals and vitamins, leading to specific micronutrient deficiencies) or both. [3, 6-8] However, the high prevalence of bacterial and parasitic diseases in

developing countries contributes greatly to malnutrition. Similarly, malnutrition increases one's susceptibility and severity of infections and is a major component of illness and death from disease. Therefore, the greatest risk factor for the burden of disease in poor nations is malnutrition. It is the direct cause of about 3L deaths per year and is indirectly responsible for about half of all deaths in young children. The severity of malnutrition is directly connected with the probability of death. The idea of using Nutraceuticals as health-promoting elements beyond its nutritional value is becoming more and more popular among the general population and the scientific community. It is defined as a substance which can be considered a food or its part which, in addition to its normal nutritional value, provides health benefits including prevention of disease or promotion of health [9].

Traditional herbal medicines and their preparations have been widely used for thousands of years in many oriental countries. However, one of the features of Eastern herbal medicine preparations is that all of the herbal remedies are extracted with boiling water during the decoction process, whether they are presented as single herbs or as collections of herbs in composite formulas [10]. In terms of world health, herbal medicines continue to play a central role in the healthcare systems of large portions of the world's population. This is particularly true in developing countries, where traditional systems of medicine have a long history of use. But one of the features of Eastern herbal medicine preparations is that, during the decoction process, all the herbs, whether they are presented as single herbs or as groups of herbs in a composite formula, are extracted with boiling water. But one of the features of Eastern herbal medicine preparations is that, during the decoction process, all the herbs, whether they are presented as single herbs or as groups of herbs in a composite formula, are extracted with boiling water. This medicine system has unique diagnosis methods and incorporates >7000 species of medicinal plants into clinical practice. [11].

The present investigation deals with the quality control profile of polyherbal formulation in the form of capsules. The formulation was the combination of different plants (Spirulina, Ashwagandha, Brahmi, Gokhru, Shankhpushpi, Mahua, Shingada, and Bael) that are traditionally used for anti-oxidant and immunomodulatory activity and also reported for a health supplement. The combination of different plants gives synergistic effects. The present study also included pharmacognostic and pharmacological evaluation of polyherbal formulation.

Materials and methods

Plant material

The whole parts of different plants (Spirulina, Ashwagandha, Brahmi, Gokhru, Shankhpushpi, Mahua, Shingada, and Bael) and roots were separately dried, powdered and then extracted to use for evaluation of various physicochemical, phytochemical and pharmaceutical parameters.

Physicochemical evaluation of extract

Determination of Ash and Extractive Values was performed. Prefilling, post filling studies were also done. Dissolution, Disintegration, and Determination of pH were also performed.

Phytochemical evaluation

Preliminary phytochemical screening of the Polyherbal formulation for the presence of various phytoconstituents was carried out using the following methods [12].

Test for alkaloids, phytosterols/steroids, phenols, tannins, flavonoids, saponin, carbohydrates, and proteins, Test for an amino acid to the known volume of test solution, 2 mL of ninhydrin solution was added and the solution was heated. The formation of violet colour indicates the presence of amino acids.

Quantitative evaluation

The estimation of total alkaloid content was done as per the gravimetric methods. Estimation of Flavonoid content done by the number of flavonoids in a sample was expressed as mg/g rutin equivalent, which was calculated by the following formula: X = (A. mo.)/ (Ao. m). The total carbohydrate content was determined as per the calorimetric method as described by Yemm and Willis (1954).[13] with a small modification, the Helaly method was followed for the saponin quantification.

Identification of active constituents carried out by TLC Method using Solvent System toluene: ethyl acetate: formic acid (7:2.5:0.5). HPLC Quantitative studies carried out**:** The chromatographic studies were performed on C_{18} analytical column (Spinchotech Pvt. Ltd. Enable). Mobile phases were prepared in closed solvent bottles and sonicated for about 20 min. Gradient mobile phase containing methanol: water (90:10) produced the best resolution of a peak at less retention time for C- Phycocyanin with a flow rate of 1.0 ml/min. The maximum absorption wavelength (λmax) of C-Phycocyanin was found to be 210 nm, hence selected as the detection wavelength for analysis [14-15].

Animal Studies were done on Swiss Albino Mice of either sex weighing 20-30 g were obtained from the Animal House (Reg. No: 92/1999/CPCSEA Dated-28/04/1999) (Reg. No-IAEC/UDPS/2017/35.), Department of Pharmaceutical Sciences, RTMNU, Nagpur and were kept under standard lab condition. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. The experimental protocol was approved by the Central Animal Ethical Committee of RTMNU Nagpur University (dated 14/08/2017).

Histopathological studies

On the 15thoperative day, the animals were anaesthetized, and tissue sampling was done. The tissues were immediately blotted, dried and fixed in formalin (10%), then dehydrated in acetone and embedded in paraffin wax for taking sections (4µm thickness) with the help of a microtome. The transverse sections of the tissues were taken. The sections were then stained with haematoxylin-eosin and processed for photo-microscopic examination using Nikon Trinocular Microscope, Model E-200 [16].

Result and discussion

Table 1: Phytochemical screening

Test	Results
Steroids	+
Saponin	+
Carbohydrates	+
Proteins	+
Amino acid	+
Tannins	+
Alkaloids	+
Flavonoids	+

Chromatographic evaluations

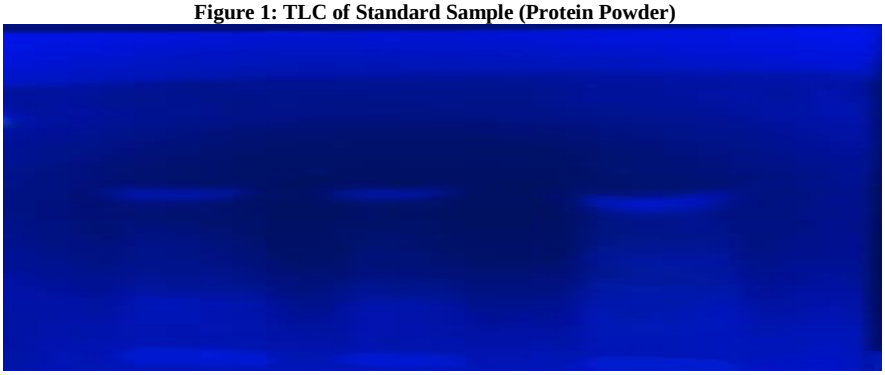
With the help of TLC and HPLC, one can easily determine the purity and quantity of a sample, identification of compounds in a mixture, separation of multi-components in pharmaceutical formulation and in cosmetic industries for the separation and identification of colours,

Preservatives and sweetening agents [17]. In the present study, TLC analysis was done for the determination of C-Phycocyanin in toluene: ethyl acetate: formic acid (7:2.5:0.5) as a solvent system. Results obtained from the TLC fingerprinting studies showed that the formulation contain a maximum amount of phytoconstituents (Figure 2) as compared with (Figure 1)as a Standard Sample.

Table 2: RF Values

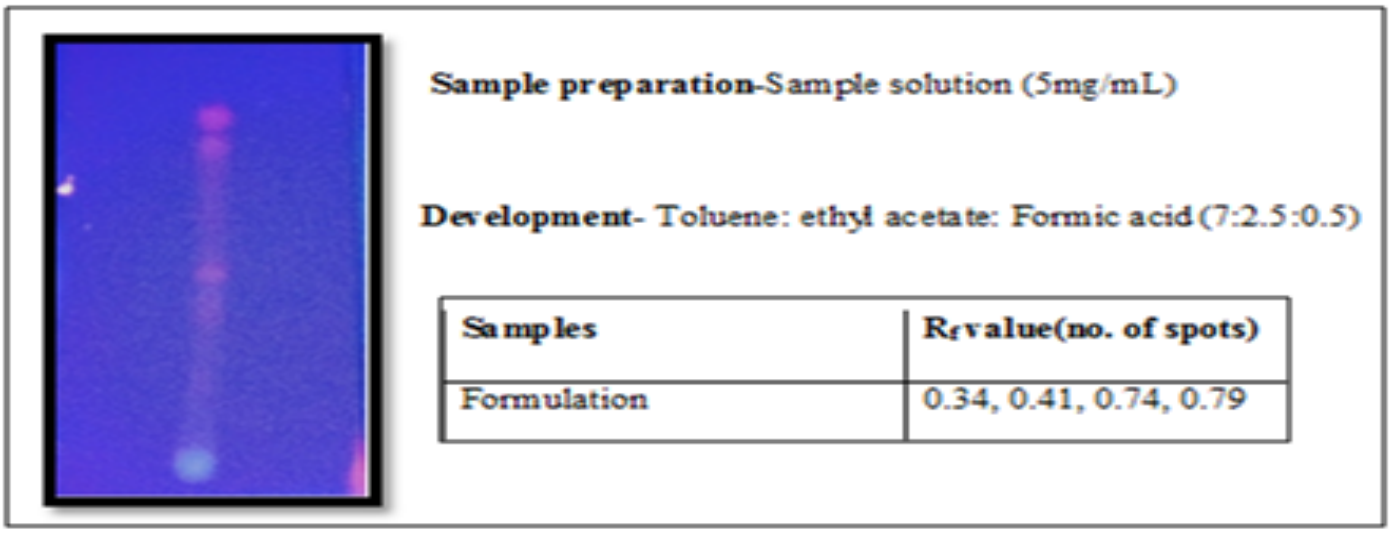
Particulars	R_f Value
Sample 1	0.58
Sample 2	0.60
Sample 3	0.57

Figure 1: TLC of Standard Sample (Protein Powder)



(1 2 3)

Figure 2: TLC Polyherbal Formulation Sample



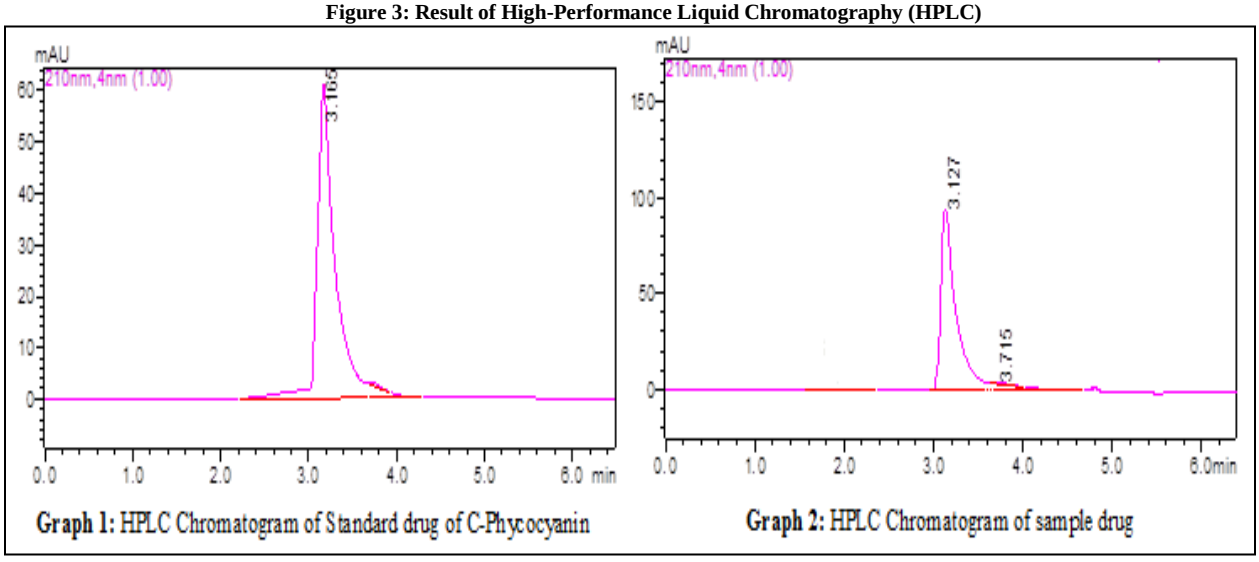
Similarly, the results obtained from the quantification studies by HPLC were shown in Table3 and Figure 2 and these results found that the C-Phycocyanin in the formulation. The concentrations of C-Phycocyanin were found to be in the polyherbal formulation. Amino acid is known to play an important role in many pharmacological conditions like inflammation, arthritis, cardiac diseases and cancer [18, 19].

Table 3: Results of HPLC Analysis

Mobile phase: Methanol: Water (90:10)

Sample ID	Concentration of Sample (mg/ml)	R.T.	Peak Area	Concentration of C- Phycocyanin (%w/w)
(Standard)	0.1	3.165	880393	-
Sample	0.1	3.127	1190700	7.39

Figure 3: Result of High-Performance Liquid Chromatography (HPLC)



Screening for Weight-Gaining Activity

The present research showed that the health supplement was found to significantly increase in the animals treated with the Polyherbal formulation compared to control. There was an increase in body weight observed in the mice, which was shown in Table 7. The results also exhibited that activity of GP and GR formulation significantly increased compared to the standard Protein powder (Prohance Mom by Lupin). The formulation significantly increased plasma antioxidant capacity by 42% compared with controls. The polyherbal formulation is used as a nutritional

supplement in many countries, it is conceivable that phycocyanin could be potentially used as a dietary supplement, becoming a helpful potential therapeutic agent in oxidative stress-induced diseases.

Table 4: Evaluation of Bodyweight

Group	Mean body weight before injection (gm)	Mean body weight on 7th day (gm)	Mean body weight on the 14th day (gm)	Mean body weight on 21stday (gm)
Control	23.42±2.17	27.81± 5.63	29.27± 5.58	31.25±4.99
Standard	24.28 ±3.44	27.08± 5.38	28.55± 4.37*	29.85±4.35*
Low dose Long term	24.55± 1.40	28.61± 2.66	29.51± 2.36	30.85±2.89
High dose Long term	24.51± 1.53	29.43± 2.29	30.27± 1.67*	31.22±1.25*
Low dose Short term	31.13± 2.26	33.75± 2.61	35.35± 3.04	-
High dose Short term	36.55± 3.18	39.32± 3.53	41.35± 3.46	-

Values are mean ± SEM of 6 parallel measurements. A statistically significant test for comparison was done by ANOVA, followed (n=6).

*P< 0.05vs Control

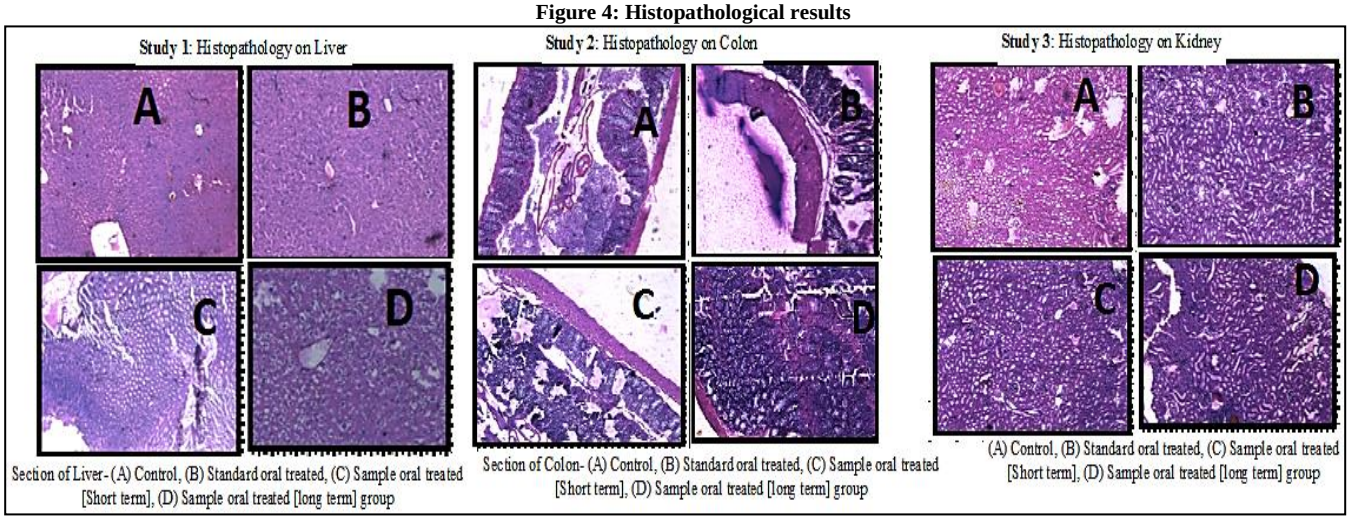
The results of histopathology clearly show that natural4). There were no changes in necrosis, the tissues were a normal structure of liver, colon and kidney being no changes in the organstructure. The results of the biochemical analysis were shown in after treatment with standard formulation and treated drug, (FigureTable5.

Table 5: Evaluation of hematology

Parameters	Control	Standard	Long term		Short term	
			Low dose	High dose	Low dose	High dose
SGPT	54.61±2.40	55.00±2.37	47.61±2.59	52.28±2.61	54.51±1.26	55.28±2.57
HB	13.31±0.85	13.74±1.25	14.20±1.34	14.52±1.89	14.91±2.18	15.50±2.54
Total LC	3600±2.38	7300±2.89	4000±3.82	4580±4.08	6500±5.27	7900±5.89
Neutrophils	3.00±0.21	4.00±0.22	5.01±0.31	3.50±0.84	5.00±0.29	5.00±0.29
Lymphocytes	94.00±0.03	92.01±0.18	92.01±0.18	90.85±1.25	90.14±1.34	93.34±2.51
Monocytes	02.00±0.10	02.01±0.11	02.00±0.10	02.00±0.10	02.00±0.10	02.00±0.10
Eosinophils	02.12±0.20	02.00±1.25	01.00±0.50	02.01±0.81	01.00±0.80	03.11±1.85
Platelet Count	815.40±0.20	1085±4.28	700.21±0.31	725.01±0.33	790.00±0.40	795±0.80
RBC	9.15±1.82	7.90±1.85	8.01±2.12	8.01±2.12	7.15±2.37	7.00±2.30

Values are mean ± SEM of 6 parallel measurements. Statistical significant test for comparison was done by ANOVA, followed (n=6)

Figure 4: Histopathological results



CONCLUSION

In the present study, physicochemical and pharmaceutical parameters were within the prescribed limits per standard values mentioned in API. The study also includes the pharmacognostic and pharmacological activity of *Arthrospira platensis* which is one of the components of polyherbal formulation. The physicochemical parameter of *Arthrospira platensis* was found to be in the prescribed range as per standard values. The phytochemical study revealed the presence of mainly alkaloids, flavonoids, tannins, alkaloids, saponins and carbohydrates which were found to be in considerable amounts in polyherbal formulation and the total polyphenol was found to be absent. The HPLC analysis confirmed the presence of C-Phycocyanin in formulation, which was found to be 7.36% w/w respectively. The polyherbal formulation of health supplement was found to significantly increase in the animals treated with the control, which was quite comparable to standard C-Phycocyanin. In conclusion, the study demonstrated a potent weight-gaining activity of polyherbal formulation. Finally, the present research work suggested that there are various synthetic drugs present for weight gain which can produce major side effects but the use of this type of polyherbal.

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