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STANDARDIZATION OF PRANAH CAPSULE AS A HEALTH SUPPLEMENT WITH ANTIOXIDANT ACTIVITY

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ABSTACT

'Ahara' comes under one of the '*Thrayopasthambhas'*. Consuming '*Satmyaaharas'* will make a person healthy, both mentally and physically. Nowadays the society gives equal importance to health supplements also. The Pranah capsule is an Ayurvedic proprietary medicine formulated by SITARAM AYURVEDA (P) LTD as a health supplement. It is a 500 mg capsule, with a combination of *Amla* (Gooseberry), *Ashwagandha* (Indian winter cherry), and *Yashtimadhu* (Licorice). To standardize the capsule formulation, phytochemical analysis, physicochemical analysis, antioxidant activity & TLC were conducted. TLC provides the presence of active ingredients in Pranah capsule which shows the same as *Amla* (Gooseberry), *Ashwagandha* (Indian winter cherry), and *Yashtimadhu* (Licorice). Phytochemical analysis showed the presence of components like tannins, coumarins, carbohydrates, sugars, alkaloids, ketoses etc. DPPH assay confirmed the antioxidant activity of *Amla*, *Ashwagandha*, and *Yashtimadhu* in Pranah capsule. Thus it is presumed that Pranah capsule can act as a good antioxidant health supplement which helps in the rejuvenating and immune booster.

KEYWORDS: Pranah capsule, *Amla, Aswagandha, Yashtimadhu*, Antioxident activity.

INTRODUCTION

Ayurveda is a traditional system of medicine developed between 2500 and 500 B.C.[1] Nowadays Ayurveda is keenly observed by the world for its various philosophical fundamentals and documentation methods. Ayurveda is referred to as the "Science of longevity", since it provides a comprehensive strategy for living a long, healthy life. It also provides plans for body renewal through diet and nutrition. Ayurveda defines health as a complete metabolic balanced state of body and mind.[2]

'*Ahara'* is considered as'*Mahoushada'* by Acharya Kasyapa.[3] Taking '*Sathmyaaharas'* will make a person healthy. Modern medicine also gives importance to health supplements nowadays. Consuming a healthy diet in the right way can lead to good health. '*Ahara'* plays a crucial role in longevity and increases mental strength while giving the body strength, complexion, and '*Oja'*. But with growing modernization, some traditional methods are being given up. Hence, modern food habits are affecting balanced nutrition.[4] There is an ever-widening gap in nutrient intake due to which normal life is no longer normal. So, we are forced to take health supplements. The purpose of health supplements is to add a little more to our normal diet so that we can stay healthy for the long term.

Any product having one or more nutritional components is accepted as a dietary supplement. A mineral, vitamin, amino acid, nutritional supplements for human use that raises the daily intake; or concentrates, metabolites, constituents, extracts, or mixtures of these substances – all these come under the category of supplements. Among these, dietary supplements that are utilized for reasons other than nutrition are nutraceuticals. Nutraceuticals are gaining a lot of attention these days because of their possible benefits in terms of nutrition, safety, and treatment. According to the latest industry research, the global nutraceuticals market is growing and is expected to reach $250 billion by 2018.[4]

The Pranah capsule is an Ayurvedic proprietary medicine formulated by Sitaram Ayurveda (P) LTD as a health supplement. The word 'Pranah' means 'Life' or 'Vitality'.[5] It is a 500 mg capsule, with a combination of *Amla* (Gooseberry), *Ashwagandha* (Indian winter cherry), and *Yashtimadhu* (Licorice). The nutritional and antioxidant qualities of these already have been the subject of extensive research.[6,7,8,9,10,11,12,13] Amla is rich in various ingredients including tannins, alkaloids, Gallic

acid, fiber, carbohydrates, vitamin C, and a rich source of antioxidants. Fruits of Amla are commonly used to treat various diseases due to their antioxidant potentiality.[12]

MATERIALS AND METHODS

Pranah capsule consists of extracts of *Amla*, *Ashwagandha*, and *Yashtimadhu*. All these ingredients were procured from All Pure Organics, Delhi, and were authenticated by a botanist, Quality assurance department, SITARAM AYURVEDA (P) LTD. A voucher specimen along with a Certificate of Analysis of the same has been kept in the Quality Control Department of Sitaram Ayurveda (P) LTD for future reference. The formulation was prepared by mixing each extract in the ratio 2:2:1 and was passed through a sieve to get a homogenous blend.

Organoleptical analysis

The extract of each ingredient was tested organoleptically by taste, color, odor, and texture.[14]

Physico-chemical Analysis

Physico-chemical investigations of formulations were carried out, including the determination of extractive values, ash values and presence of phytochemical properties.[14]

Table 1: Shows the features of the Pranah capsule.

Avg.wt.of a capsule	650 mg± 5 %
Disintegration Time	NMT 30 min
LOD	NMT 7%

Thin Layer Chromatography (TLC)[15]

Preparation of Test solution: Refluxed 1 g powdered drug with 10 ml of ethanol for 15 minutes. Filtered and the filtrate was evaporated to dryness. Dissolved the residue in 2 ml ethanol.

Preparation of Solvent system: Chloroform: Methanol (9:1)

Procedure: To a clean coupling jar, pipetted out chloroform and methanol with a ratio of 9:1 respectively. The extract was spotted 1 cm above the base of the silica plate with a capillary tube. The plate inside the coupling jar and solvent system was allowed to move till the solvent front without any disturbances.

Preparation of spraying reagent

Spraying reagent: Anisaldehyde sulphuric acid.

Mixed 0.5 Anisaldehyde with 10 ml glacial acetic acid followed by 85 ml methanol and 5 ml concentrated sulphuric acid.

Sprayed the plate with 10 ml of Anisaldehydesulphuric acid and heated it at 120˚C for 5-10 minutes. Evaluated the plate in UV 365 light.

Evaluation: The spots or bands appeared on the plate. Retention factor (Rf) values were calculated for each band.

Rf value = Distance traveled by solute/Distance traveled by the solvent

Antioxidant analysis

Antioxidant activity was measured using UV-Visible Spectrophotometer.[16]

Instruments

UV-Visible Spectrophotometer: CKL/ANL/E-023
Balance: CKL/ANL/E-001

Reagents

1,1 Diphenyl-2-picryl hydroxyl(DPPH) Methanol.

Principle

The free radical scavenging activity was determined based on the activity of the stable free radical (DPPH) with antioxidants in organic/aqueous media resulting in thebleaching of DPPH due to its quenching by the interaction with the analyses. The decrease of absorbance of DPPH compared to the blank was measured spectrophotometrically at 516nm related to the concentration of antioxidants in the test solution.

Preparation of Standard solution

For the standard preparation, 0.066mM of the solution was prepared by weighing 2.6mg of DPPH in a 100 ml volumetric flask, sonicate and making up the volume to the mark with methanol.

Sample preparation

For the sample, 25.4 mg Pranah capsule powder is prepared in 25 ml methanol. From that, 4 ml was taken and made up to 25 ml.
Control sample -1.5 ml DPPH reagent+1.5 ml methanol

1. 1.5 ml DPPH+ 0.1 ml sample and made up to 3.0 ml with methanol
2. 1.5 ml DPPH+ 0.2 ml sample and made up to 3.0 ml with methanol
3. 1.5 ml DPPH+ 0.3 ml sample and made up to 3.0 ml with methanol

The reaction mixture was incubated at 37^{o}c for 30 minutes. Absorbance was measured spectrometrically at 516 nm.

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

A graph is plotted with concentration on the X axis and DPPH activity on the Y axis.
On the basis of this, IC 50 value was calculated.

RESULT

The following results were obtained through the various Standardization procedures.

Macroscopic Analysis by the Organoleptic method

The Organoleptic analysis is based on color, taste, texture, and odor.

Fig. 1: Organoleptical identification of the Pranah capsule.

Table 2: Organoleptical analysis of the Pranah capsule.

Sl. No.	Organoleptic examination	Extract of Pranah
1.	Color	Yellowish brown
2.	Texture	Fine powder
3.	Taste	Astringent, bitter
4.	Odor	Aromatic

Physicochemical analysis

The result obtained through the physicochemical analysis of Pranah capsule is tabulated in Table no 3.

Table 3: Physicochemical analysis of Pranah capsule.

Sl. no	Parameters	Result
1	Foreign Matter	NIL
2	Total Ash	10.10%
3	Acid insoluble Ash	1.5%
4	Water Soluble Extractive	87.59%
5	Alcohol Soluble Extractive	13.22%

Phytochemical analysis

Table 4: Phytochemical analysis.

Sl No.	Organic Phytochemical constituents	Name of the test conducted	Water Extract	Alcoholic Extract
1.	Carbohydrate	Molisch's test	+	-
2.	Sugar	Benedict's test	+	+
3.	Ketose	Seliwanoff's test	+	-
4.	Protein	Biuret test	-	-
5.	Starch	K I test	-	-
6.	Glycoside	Keller killiani test	-	-
7.	Steroid	Salkowski test	-	-
8.	Terpenoid	Salkowski test	-	-
9.	Flavonoid	Alkaline reagent	-	+
10.	Phenol	Phenol reagent test	-	-
11.	Saponin	Foam test	+	-
12.	Alkaloid	Wagner reagent	-	+
13.	Tannin	Ferric chloride test	-	+
14.	Coumarin	NaOH test	-	+

Thin layer chromatography

Figure -2, 3, and 4 - Chromatogram of *Ashwagandha*, *Amla*, and *Yashtimadhu* respectively in comparison with the chromatogram of Pranah extract.

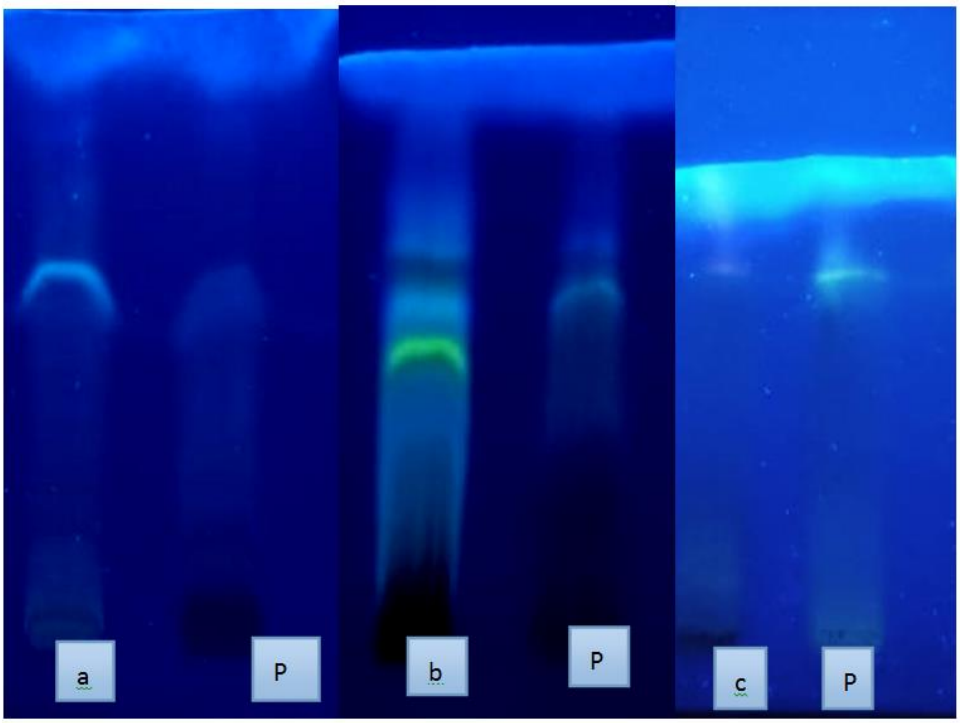

Fig: 2 **Fig: 3** **Fig: 4**

Figure 2: a: *Ashwagandha* extract, p: Pranah capsule,
Figure 3: b: *Yashtimadhu* extract, p: Pranah capsule,
Figure 4: c: *Amla* extract, p: Pranah capsule.

Table 4: Comparison of Rf values of *Ashwagandha* & Pranah.

Spots	Rf Value						Mean	
	I		II		III			
	Ashwagandha	Pranah	Ashwagandha	Pranah	Ashwagandha	Pranah	Ashwagandha	Pranah
A	0.54	0.53	0.52	0.53	0.51	0.52	**0.50**	**0.50**

Table 5: Comparison of Rf values of *Yashtimadhu* & Pranah.

Spots	Rf Value						Mean	
	I		II		III			
	Yashti	Pranah	Yashti	Pranah	Yashti	Pranah	Yashti	Pranah
A	0.40	-	0.41		0.40		**0.40**	-
B	0.48	0.48	0.47	0.48	0.48	0.47	**0.48**	**0.48**
C	0.57	0.58	0.58	0.57	0.58	0.58	**0.58**	**0.58**

Table 6: Comparison of Rf values of *Amla* & Pranah.

Spots	Rf Value						Mean	
	I		II		III			
	Amla	Pranah	Amla	Pranah	Amla	Pranah	Amla	Pranah
A	0.62	0.63	0.61	0.62	0.60	0.61	**0.61**	**0.62**

Table 7: Antioxidant activity value of Pranah capsule.

Parameters	Unit	Result	Test Method
UV Spectrometer Analysis - DPPH			
Antioxidant Activity IC 50 value of Pranah-Extract	mg/kg	16.11	CKL/ANL/UV .OO4

DISCUSSION

Increasingly people are realizing that the imbalance between the body's pro- and anti-oxidant homeostatic mechanisms is the root cause of most illnesses. A pro-oxidant state in body predominates because of either an increase in free radical production or inadequate quenching/scavenging of these radicals. Free radicals are the building blocks of all metabolic reactions and are vital to the aerobic cycle and human metabolism. The body produces them continuously through processes

including breathing and some cell-mediated immunological responses. The body generates free radicals in a dynamic balance and antioxidants are produced to scavenge and/or quench these free radicals and protect the body from their harmful effects. As a result, it has been suggested that oxidant status in humans serves as a valuable tool for evaluating the risk of oxidative damage.[12] It represents the dynamic balance between pro-oxidant factors and antioxidant response.

A combination of herbal extracts with proven antioxidant properties is found in Pranah capsules.[12,13,17] *Emblica officinalis*, also known as *Amla*, is a significant *rasayana* in Ayurveda medicine.[18] Amla is an essential component of several Ayurvedic medicines, such as *Chyawanprash*, a general tonic for persons of all ages that promotes general physical and mental well-being.[19] According to the two main classic texts on Ayurveda, Charak Samhita & Sushruta Samhita, amla is regarded as "one of the best rejuvenating herbs". Ascorbic acid and other phytoconstituents such as gallic acids, chebulic acid, ellagic acid, quercetin, rutin, linoleic acid, and emblicanin A and B are abundant in *Emblica officinalis*. Scientific research has already demonstrated the antioxidant capacity of the herb in the hepatic system, skin, renal system, cardiovascular system, diabetes, and cancer/ tumor cells.[19]

In Ayurveda, *Ashwagandha* is classified as a *rasayana*. In addition to *rasayana* it is being used to enhance mental and physical well-being, offering prevention of illness, and delay in the aging process. Botanically named as *Withania. somnifera,* it is used as a dietary supplement and the decoction of its root is used as a nutrient and health restorative for pregnant and the aged. *W. somnifera* extract is a complex mixture of numerous distinct phytochemicals, including flavonoids and phenolic compounds. On the other hand, withanolides are considered to be responsible for the pharmacological activity of *W. somnifera* roots.[13] The phenols, flavonoids, anthocyanins, alkaloids, tannins, and steroidal lactones (withanolides) are responsible for its antioxidant properties.[20]

A well-known *rasayana* drug, *Yashtimadhu*/licorice-botanically named as *Glycirrhiza glabra*, is a source of proteins, amino acids, polysaccharides and simple sugars as well as mineral salts (calcium, phosphorus, sodium, potassium, iron, magnesium, silicon, selenium, manganese, zinc, and copper), pectins, resins, starches, sterols, and gums. It is also described in Ayurvedic literature.[18] It has been reported that glycosides, estrogens, tannins, phytosterols (sitosterol and stigmasterol), coumarins, and vitamins (B1, B2, B3, B5, E, and C) are also present in it. Numerous biologically active phytoconstituents have been identified from licorice-primarily flavonoids, triterpenes, and saponins (which give it its sweet taste).[21] Glabridin, hispaglabridin A, and B hydroxymethylglabridin are phenolic compounds mainly responsible for the antioxidant activity of licorice which has proven their action of protection of low-density lipoprotein from oxidation. The presence of Licochalcones B and D in *G. glabra* have also got validated for their anti-oxidant activity and potential inhibition of the microsomal lipid peroxidation.[21]

Blending multiple drugs to create a Pranah pill does not alter the presence of the active principles found in the drug extract. The Withaferin spot at Rf 0.50 present in Pranah, was prepared using the three drugs together.[14] The chromatograms of *Yashtimadhu* and Pranah shows similar bands at Rf 0.48 and 0.58 in the mobile phase of Toluene: Ethyl acetate: Acetic acid (5:4.2:0.8). Three spots were present in the *Yashtimadu* extract but in Pranah, 2 major spots were only seen. The missing of one spot at Rf 0.40 in Pranah is a possibility that a new compound has formed by the combination of other drugs.[22] In Figure 4, in the Amla extract the main component of Gallic acid is present at Rf 0.61 and is present in Pranah at Rf 0.62.[23]

DPPH assay was used for the determination of the antioxidant activity of Pranah capsule. Antioxidant capacities of the extracts were expressed in terms of IC50 value of the extracts and low IC50 value corresponds to a high antioxidant capacity.[13] Here, the DPPH assay confirmed the antioxidant activity of *Amla*, *Ashwagandha* and *Yashtimadhu* in the capsule. Hence, it is suggested that Pranah capsule will be a good rejuvenating formulation and an immune booster as the ingredients in it are proven for the same.

CONCLUSION

The Pranah capsule is an Ayurvedic proprietary medicine formulated by Sitaram Ayurveda (P) LTD as a health supplement. It is a combination of *Amla* (Gooseberry), *Ashwagandha* (Indian winter cherry), and *Yashtimadhu* (Licorice). The current article is aimed to standardize the formulation with its antioxidant activity and the study results proved the same.

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