Comparative Phytochemical and Antioxidant Activity of *Ashtavarga* herbs with their *Bhavprakasha Nighantu* Substitute Herbs

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Abstract- *Ashtavarga* is a group of eight herbs which are considered very good *rasayana* (potent tonic) to rejuvenating, health promoting, strengthening the immune system, antioxidant and have immense cell regeneration capacity and are commonly used in promoting body health, healing fractures, seminal weakness, fever, abnormal thirst, diabetic conditions and also as a cure for Tridoshas in human health. *Ashtavarga* herbs are commonly used in different dosage forms, e.g. as Avaleha (Chyavanprasha), Tonic, *Taila* (medicated oil*)*, *Ghritam* (medicated clarified butter), *Churana* (powder) etc. In this research paper we have tried to find out the antioxidant activity and phyto-chemical screening of Ashtavargra herbs in comparison to substitute herbs recommended by the renowned Ayurvedic Text "*Bhavprakasha Nighantu*". The results of this research study shows that the substitute herbs recommended by *Bhavprakasha Nighantu* show greater antioxidant activity as compare to *Ashtavarga* herbs. *Ashtavarga* herbs shows maximum antioxidant activity of 59.82% at the concentration of 700mcg/ml whereas substitute herbs show 89.09% at only 180 mcg/ml which is very less as compared to the *Ashtavarga* herbs concentration. In case of phytochemical screening we find that the maximum total tannins and total bitters found in Varahikand (*Dioscorea bulbifera L.*) and Jeevak (*Microstylis musifera*) respectively. Similarly, minimum Total Tannins and Total Bitters found in Riddhi (*Habenaria intermedia)* and *Shatavari* ((*Asparagus Racemosus)* respectively.

Keywords: *Ashtavarga* , Antioxidant activity, Tannins, Bitters, DPPH.

Introduction

The *Ayurveda* is the ancient science of life. During starting development phase of *Ayurveda*, *Ashwani Kumars*, a reputed *Ayurvedic* wonder healers saw the old and sleazy body of *Chayavan Rishi*. They decided to formulate a medication to rejuvenate the body of their sage Chayavan Rishi. *Ashwani Kumars made* a unique preparation with *Ashtavarga herbs* (a group of eight medicinal plants) to rejuvenating the body of *Rishi Chayavan* and they successfully restored his body. Therefore, due to the name of *Rishi Chayavan* the preparation was called as *Chayavanprash*. These medicinal plants started fading away over the several centuries. All *Ashtavarga* herbs have their natural habitats in the North- West Himalaya. *Ashtavarga* herbs are considered as a very good *rasayana* to rejuvenating, health promoting, streng-

then the immune system, antioxidant and have immense cell regeneration capacity and are commonly used in promoting body health, healing fractures, seminal weakness, fever, abnormal thirst, diabetic conditions and also as a cure for *vata*, *pitta*, *rakta doshas*. *Ashtavarga* herbs are commonly used in different dosage forms, e.g. *Chyavanprasha*, Tonic, *Taila* (oil*)*, *Ghritam* (medicated clarified butter), *Churana* (powder). The *Dhanvantri Nighantu* (ancient text) has the description of highest number of names and pharmacological properties for *Ashtavarga* herbs. *Bhava Prakash Nighantu*, *Shaligram Nighantu* and *Rajnighantu* also have *Ashtavarga* herbs names and their therapeutic properties[1-11]. Due to difficulty to arrange *Ashtavarga* herbs, B*hav Prakash Nighantu* had described their substitute herbs given in **Table-1**. Therapeutic application of *Ashtavarga* herbs and their substitutes as per *Bhavprakash nighantu* is given in **Table-2**.

Table-1 *Ashtavarga* **herbs and their** *Bhavprakasha Nighantu* **recommended substitute herbs**

SN	*Ashtavarga* **Plants**	**Substitute Plants**
01.	*Meda (Polygonatum airrhifolium Royle & other species)*	Shatavari ((*AsparagusRacemosus)*
02.	*Maha Meda (Polygonatum airrhifolium Royle & other species)*	
03.	Jeevak (Microstylis musifera Ridley; Lipasis rostrata Rehd))	Vidarikand (*Pueraria tuberosa*)
04.	*Rishbhak* (Microstylis wallichii, Linn; Lipasis rostrata Rehd)	
05.	Kakoli *(Roscoea procera Wall; Lillium polyphyllum D. Don))*	Aswagandha (*Withania somnifera*)
06.	Kshirakakoli *(Roscoea procera Wall; Lillium polyphyllum D. Don))*	
07.	Riddhi (*Habenaria intermedia D. Don; H. edgeworthii Hook, H. acuminate Thw; H. goodyeroides D. Don)*	Varahikand (*Dioscorea bulbifera*)
08.	*Vriddhi* (*Habenaria intermedia D. Don; H. acuminate Thw; H. goodyeroides D. Don)*	

Table-2 Therapeutic applications of *Ashtavarga* herbs **and their** *Bhavprakasha Nighantu*

SN	*Ashtavarga* **Plants**	**Therapeutic applications**
01.	*Meda (Polygonatum airrhifolium*	Veerya vardhak, Dugdh tatha Kaf ko badhane wale, Dhatu vardhak, Sheetal, Pitt, Raktsambandhi dosh, Vayu tatha juvar ko door karne wala.
02.	*Maha Meda (Polygonatum airrhifolium)*	
03.	Jeevak (Microstylis musifera	Balkarak, Sheetvirya, Guru, Shukra tatha kaf ke vardhak, Madhur rasyukt, Pitt, Dah, Raktdosh, Krushta, Vaat evam Kshe rog.
04.	*Rishbhak* (Microstylis wallichii)	
05.	Kakoli *(Roscoea procera)*	Sheetal, Shukra vardhak, Madhur, Guru, Dhatu vardhak, Vaat, Dah, Raktpit, Sosh evam jevar nashak.
06.	Kshirakakoli *(Roscoea procera)*	
07.	Riddhi (*Habenaria intermedia)*	Balkarak, Tridosh, Shukrvardhak, Madhur ras yukt, Pak me guru, aur pranprad,

		aishvaryajanak, Murchha avam Raktpit ka nash karne wali
08.	*Vriddhi* (*Habenaria intermedia)*	Garbhprad, Sheetal, Dhatuvardhak, Madhur ras yukt, Veerya vardhak, Raktpit, kshat, Kash tatha Ksha ko door karne wali.
	Ashtavarga	**Substituted herbs**
01.	Shatavari ((*AsparagusRacemosus)*	Madhur, Sheet, Guru, Snehan, Stanyajanan, Mutrajanan, Shukrajanan, Balya, Virashya, Vayasthapan, Chakushya, Agnivardhak, Alpsangrahak avam Tridoshghan.
02.	Vidarikand (*Pueraria tuberosa*)	Stanyajanan, Mutrajanan, Poshtik, Balya, Dugdh vardhak, Shoth par peeskar bandhte hai,
03.	Aswagandha (Withania somnifera)	Ushna, Madhur, Bhraney, Balya, Rasayan, Vrashya, Shothhar, Ksha, Balshos, Sukhandi, Vardhakya, Poshtik, Ishtiryo ke Katishool avam Shwed pradar me.
04.	Varahikand (*Dioscorea bulbifera*)	Raktsanghrahak, Grahi, Charm rog.

Material and Methods

Ethyl acetate, Methanol, Potassium ferricyanide, Ferric Chloride, Tannic acid, 1,1-diphenyl-2-picrylhydrazyl (DP PH) (Sigma-Aldrich, Germany), and all reagents are analytical grade.

Sample Preparation- all herbs are collected from approved vendors and authenticated by Dr. Mayaram uniyal. Clean all herbs with running tap water, dry them at 30 °C in hot air oven after drying make powder with grinder. This powder without any sieving will take for all analysis.

Antioxidant Activity

DPPH radical scavenging activity Using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), Brand Williams et al methodology slightly modifies is utilized to evaluate the actions of DPPH scavenging with methanol extract of *Ashtavarga* herbs and their substitutes. The different concentration of *Ashtavarga* herbs and their substitutes has prepared (30 mcg/ml to 700 mcg/ml). Each concentration was measured in triplicate. The 3.0ml of each sample solution is mixed with 1.0ml of 0.1mM DPPH in methanol. After shaking keep the solution at room temperature for exactly 30 min. in a light protected area. The absorbance was taken at 517nm with UV 1800 Made by Shimadzu. The difference of absorbance obtained from the sample and control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radicals. The following formula was utilized to compute the degree of DPPH radical scavenging activity.

Inhibition % = Ac-As/Ac×100

Where, Ac is the absorbance of the control,
As is the absorbance of the sample.

Total Bitter Determination-Weigh about 3 g of sample powder into round bottom flask add 100 ml methanol and reflux on water bath at 80 ± 2°C for 1 hour, remove the flask and allow to cool, filter the supernatant into 250 ml beaker through whatman filter paper number 1. The residue will again reflux with 50 ml methanol, this process will continue till the extract become colorless, collect all the filtrate in the same beaker. Beaker now keep on water bath for evaporation

to get thick paste, remove the beaker and add 50 ml hot water dissolve the paste and transfer into the separating funnel and add 100 ml ethyl acetate, shake gently and allowed to separate the ethyl acetate layer. Transfer the ethyl acetate layer into pre-weighed beaker with the help of whatman paper 1. The aqueous layer will again reflux with 50 ml ethyl acetate, this process will continue till the ethyl acetate layer become colorless. Transfer all ethyl acetate layers intosame beaker, keep it on water bath for evaporation and finally keep in oven at 105 °C for 2 hours take the weigt and calculate the total bitter as per below formulae.

% Total bitter = (Weight of beaker after drying- weight of empty beaker)/weight of sample *100

Total Tannins Determination

Sample preparation- Weigh about 100 mg sample powder into 250 ml round bottom flask and 100 ml water and reflux on water bath at 100 °C for 1 hour, remove the flask and allow to cool and filter into 100 ml volumetric flask, make up the volume up to the mark with water, shake and filter through whatman filter paper (working sample solution) this filtrate will use for sample preparation for taking absorbance from UV spectroscopy.

Standard preparation- weigh 100 mg Tannic acid into 100 ml volumetric flask and add 50 ml water, sonicate for 5 minutes make up the volume with water (Stock Standard). Take 1 ml stock standard into 100 ml volumetric flask and add water to make the volume up to the mark (working standard solution), this standard solution is used for taking absorbance from UV spectroscopy[12-16].

Reagent Preparation

1. **1%Potassium Ferricyanide solution-** Take 1 g of potassium ferricyanide into 100 ml volumetric flask add about 50 ml of water dissolve by sonication for 10 minutes and finally make up the volume up to the mark and filter through whatman filter paper 1.
2. **1% Ferric chloride solution-** Take 1 g of ferric chloride into 100 ml volumetric flask add about 50 ml of water dissolve by sonication for 10 minutes and finally make up the volume up to the mark and filter through whatman filter paper 1.

Preparation for UV Spectro-photometer reading

Reagent Preparation- add 1 ml of 1% potassium ferricyanide and 1 ml of ferric chloride reagents into 10 ml volumetric flask and make up the volume up to the mark with water.

Standard preparation- take 1 ml of working standard solution into 10 ml of volumetric flask add 1 ml of potassium ferriccyanide and 1 ml of ferric chloride solution and make up the volume with water. After 30 minutes of reagent addition take the reading against reagent at 720 nm.

Test preparation- take 0.2 ml of working sample solution into 10 ml of volumetric flask add 1 ml of potassium ferriccyanide and 1 ml of ferric chloride reagent and make up the volume with water. After 30 minutes of reagent addition take the reading against reagent at 720 nm.

Test Blank preparation- take 0.2 ml of working sample solution into 10 ml of volumetric flask, make up the volume

with water. After 30 minutes take the reading against water at 720 nm.

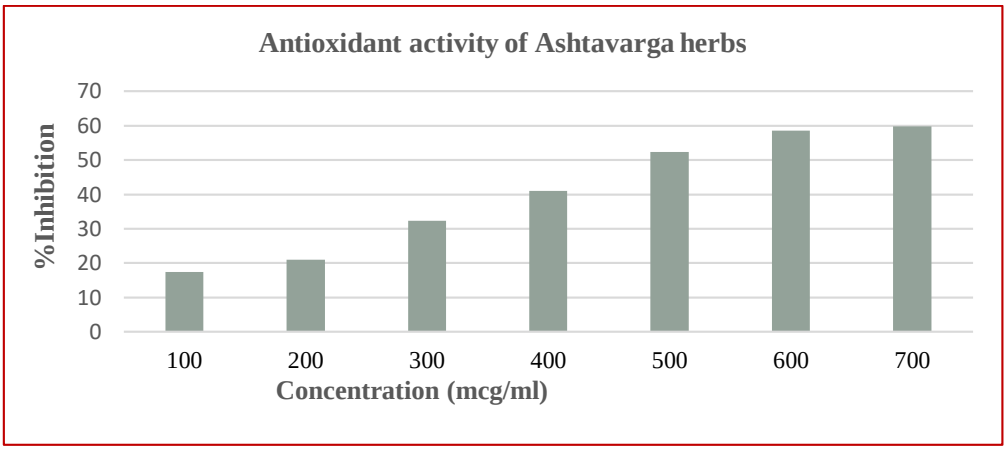
Calculate the total tannins as per given formula-

% Total Tannins	=	Absorbance of sample - absorbance of sample blank	Weight of standard	1	volume of std taken	total volume of sample	% Purity of std
		Absorbance of std	100	100	volume of test taken	weight of sample	

Result and Discussion

Table-3 Antioxidant activity of *Ashtavarga* herbs

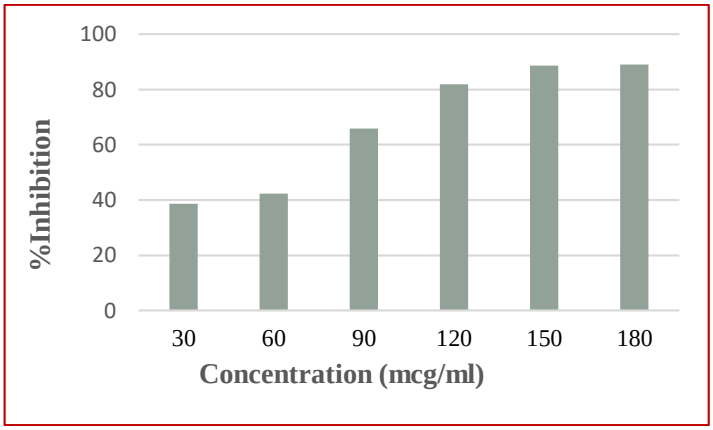
SNo.	Concentration	Plant extract (% Inhibition)
1	100 mcg/ml	17.46
2	200 mcg/ml	20.96
3	300 mcg/ml	32.31
4	400 mcg/ml	41.04
5	500 mcg/ml	52.40
6	600 mcg/ml	58.51
7	700 mcg/ml	59.82



Graph-1 Antioxidant activity of *Ashtavarga* herbs

Table-4 Antioxidant activity of *Bhavprakash nighantu* *Ashtavarga* substitute herbs

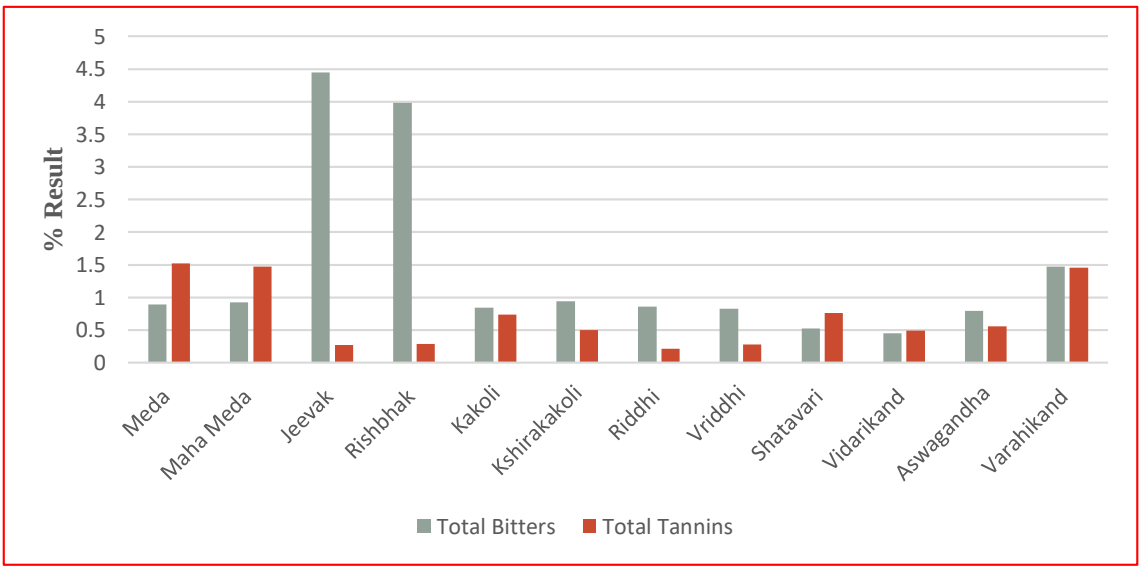
SN	Concentration	Plant extract (% Inhibition)
1	30 mcg/ml	38.63
2	60 mcg/ml	42.27
3	90 mcg/ml	65.90
4	120 mcg/ml	81.81
5	150 mcg/ml	88.63
6	180 mcg/ml	89.09



Graph-2 Antioxidant activity of Substitute herbs

Table-5 Phytochemical screening of *Ashtavarga* herbs and their substitute

SN	***Ashtavarga*** **Plants**	**% Total Bitter**	**% Total Tannins**
01	*Meda (Polygonatum airrhifolium*	0.889	1.42
02	*Maha Meda (Polygonatum airrhifolium)*	0.924	1.45
03	Jeevak (Microstylis musifera	4.444	0.27
04	*Rishbhak* (Microstylis wallichii)	3.985	0.29
05	Kakoli *(Roscoea procera)*	0.841	0.74
06	Kshirakakoli *(Roscoea procera)*	0.945	0.50
07	Riddhi (*Habenaria intermedia)*	0.860	0.21
08	*Vriddhi* (*Habenaria intermedia)*	0.825	0.28
Substitute Plants			
01	Shatavari ((*AsparagusRacemosus)*	0.526	0.76
02	Vidarikand (*Pueraria tuberosa*)	0.451	0.49
03	Aswagandha (Withania somnifera)	0.797	0.56
04	Varahikand (*Dioscorea bulbifera L.*)	1.47	1.46



Graph-3 Phytochemical Screening of *Ashtavarga* Plants and their substitutes

Discussion

From the above tables and graphs, the *Ashtavarga* substitute herbs recommended by *Bhavprakasha Nighantu* shows greater antioxidant activity as compare to *Ashtavarga* herbs. Ashta-varga herbs shows maximum anti-oxidant activity of 59.82% at the concentration of 700mcg/ml whereas substituted herbs shows 89.09% at 180 mcg/ml which is very less as compare to *Ashtavarga* herbs concentration. In case of phytochemical screening we analysed individual herbs and find that the maximum Total Tannins and Total Bitters found in Varahikand (*Dioscorea bulbifera*) and Jeevak (Microstylis musifera) respectively. The minimum Total Tannins and Total Bitters found in Riddhi (*Habenaria intermedia)* and Shatavari ((*Asparagus Racemosus)* respectively.

Conclusion

This study concludes that the substitute herbs of *Ashtavarga* recommended by *Bhavprakash nighantu* is comparable with *Ashtavarga* herbs in their phytochemical screening. In case of antioxidant activity the substitute herbs are more effective as compared to *Ashtavarga* herbs and hence using of these herbs in polyherbal formulation like Chavyanprash is scientifically justified.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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