International Journal of Medical & Pharmaceutical Sciences
DOI: http://dx.doi.org/10.31782/IJMPS.2017.7201

Research Article

IJMPS
Section: Healthcare
IC Value: 6.995

Physicochemical Evaluation of Formulation of Ashwagandharista Prepared by Traditional and Modern Method

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ABSTRACT

Introduction: Ashwagandharista is an Ayurvedic polyherbal hydro-alcoholic preparation and is used as Rasayana. Rasayanas are used to promote health and longevity by increasing defence against disease. The chief ingredient of Ashwagandharista is roots of ashwagandha and has many actions on body like anti-ageing, adaptogenic, immunomodulatory, anxiety, depression, stress, cardiovascular protection etc.

Aim: The aim of this study is standardization and comparative evaluation of Ashwagandharista prepared by traditional and modern method.

Objective: The objective of this research is standardization and comparative evaluation of Ashwagandharista to improve the specific method and reduce the time-consuming process in preparation of Ashwagandharista.

Methodology: The methods used for the physicochemical evaluation and standardization of formulation of Ashwagandharista prepared by traditional and modern method were TLC and HPTLC.

Results: The physicochemical evaluation of formulation of Ashwagandharista prepared by traditional and modern method were comparatively evaluated and results found to be under limits as well as safe and effective of both the formulation of Ashwagandharista.

Conclusion: From this study, we conclude that the time required for formulation of Ashwagandharista prepared by different methods (Traditional & Modern method) can be reduced up to 20 % by modification in the process without affecting quality and efficacy of formulation.

Key Words: Ashwagandharista, Traditional and Modern Method, Physicochemical evaluation, Time-consuming process, standardization and comparative evaluation, Medicinal values

INTRODUCTION:

Ashwagandha has long been considered as an excellent rejuvenator, a general health tonic and a cure for a number of health complaints. It is a sedative, diuretic, anti-inflammatory and generally respected for increasing energy, endurance and acts as an-adaptogen that exerts a strong immunostimulatory and an anti-stress agent. Ashwagandha is taken for treating cold and coughs, ulcers, emaciation, diabetes, conjunctivitis, epilepsy, insomnia, senile dementia, leprosy, Parkinson's disease, nervous disorders, rheumatism, arthritis, intestinal infections, bronchitis, asthma, impotence and a suppressant in HIV/AIDS patients.2

Ashwagandharista prepared using Ashwagandha is an Ayurvedic polyherbal hydro-alcoholic preparation and is used as Rasayanas; Rasayanas are used to promote health and longevity by increasing defence against disease. The chief ingredient of Ashwagandharista is roots of ashwagandha Withania somnifera, commonly known for its usefulness in the treatment of hypercholesterolemia, arthritis in combination with other drugs, is also credited to be hypoglycemic and diuretic.1 The pharmacological effect of the roots of Withania somnifera is attributed to withanolides, a group of steroidal lactones.

Aim/Objective: The present study was undertaken to verify the efficacy of both the test formulations of Ashwagandharista

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ISSN: 2231-2188 (Print) **ISSN:** 2231-685X (Online)

Received: 05.02.2017 **Revised:** 10.03.2017 **Accepted:** 04.05.2017 **Published:** 10.08.2017

prepared by traditional and modern methods respectively in the presence of self-generated alcohol which helps in the faster absorption of biologically active compounds as tannins, flavonoids and steroidal saponins, glycowithanolides which by their chemical nature are antioxidants, might contribute to the prevention of cardiac diseases as hypertension by acting as diuretics.

Methods Used: In the present study, Ashwagandharista was prepared by traditional method and was standardized by TLC method. Physicochemical and phytochemical analysis was performed to confirm the chemical constituents from Ashwagandha root. However present study has few lacunas, formulation should be standardized by HPTLC methods.

METHODOLOGY

Preparation of Ashwagandharista-T & M

Preparation of Kadha (Method-A):

Coarsely powdered Ashwagandha roots (Withania somnifera) with prescribed ingredients (table 1) were placed in polished vessel of brass along with prescribed quantity of water, and allowed to steep. After 8 hrs of steeping, this material was warmed at medium flame until the water for decoction reduced to one eighths of the prescribed quantity, then the heating was stopped and it was filtered in cleaned vessel.7

Table 1. Ingredients of Ashwagandharista (Kadha)7.

Name of Drugs	Plant part	Quantity
Ashwagandha	Roots	2.4 Kg
Kali musali	Roots	960 g
Manjistha	Roots	480 g
Haritaki	Fruits	480 g
Haridra	Rhizomes	480 g
Daruharidra	Stem	480 g
Yasti	Roots	480 g
Rasna	Roots	480 g
Vidari	Roots	480 g
Arjuna	Steam bark	480 g
Mustaka	Rizomes	480 g
Svet Chandan	Heartwood	384 g
Rakta Chandan	Heartwood	384 g
Chitraka	Roots	384 g

Preparation of Kadha (Method-A):

Coarsely powdered Ashwagandha roots (Withania somnifera) with prescribed ingredients (table 2) were placed in polished vessel of brass along with prescribed quantity of water, and allowed to steep. After 8 hrs of steeping, this material was warmed at medium flame until the water for decoction reduced to one eighths of the prescribed quantity, then the heating was stopped and it was filtered in clean vessel.7

Table 2. Ingredients of Ashwagandharista-T and M (Prakshepa Dravyas)7.

Name of Drugs	Plant part	Quantity
Jaggery (gud)	-	5 kg
Dhataki / Yeast	Flower	768 g
Sonth	Rhizome	96 g
Marica	Fruits	96 g
Pippali	Fruits	96 g
Elaichi	Seeds	192 g
Tejpatra	Leaf	192 g
Nagakesara	Stmn	96 g
Priyangu	Flower	192 g

Evaluation formulation:

Determination of pH:

Taken both 40 ml of sample (Ashwagandharista) in beaker and pH of sample was determined at 27°C using digital pH meter.5

Determination of Alcohol:

This method was intended only for certain liquid preparations containing alcohol. Where the preparation contains dissolved substances that may distil along with alcohol Method I must be followed.

Apparatus:

The apparatus consists of a round-bottom flask fitted with a distillation head with a steam trap and attached to a vertical condenser. A tube is fitted to the lower part of the condenser and carries the distillate in to the lower part of a 100 ml volumetric flask. the volumetric flask is immersed in a beaker containing a mixture of ice and water during the distillation. A disc with a circular aperture, 6 cm in diameter, is placed under the distillation flask to reduce the risk of charring of any dissolved substances.

Method:

A 25 ml of the formulation of Ashwagandharista being examined, accurately measured at 24.9°C to 25.1°C, to the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100 ml volumetric flask. The temperature was adjusted to 24.9°C to 25.1°C. Determine the relative density at 24.9°C to 25.1°C. The values indicated in table are multiplied by 4 in order to obtain the percentage of alcohol by volume contained in the preparation5.

Determination of total solids:

Transferred accurately 50 ml of both sample of aristha to an evaporable dish which has been dried to a constant weight and evaporate to dryness on a water bath, then dried at 105°C for 3 hours. After cooling the dish containing the residue in a desiccator for 30 min, weighed it immediately.5

Weight Per Millilitre:

The weight per milliliter of a liquid is the weight in gm of 1ml of a liquid when weighed in air at 25°C.

Method:

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25°C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C when weighed in air of density 0.0012 g per ml, is 0.99602 g. calculate the capacity of the pycnometer, adjust the temperature of the substance to be examined, to about 20°C and fill the pycnometer with it adjust the temperature of the filled pycnometer to 25°C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in gm, of the quantity of the liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.7

Determination of Specific gravity:

The specific gravity of a liquid is the weight of given volume of the liquid at 25°C compared with the weight of an equal volume of water at the same temperature of weighing being taken in air.

Method:

Proceed as described underweight per ml. obtain the specific gravity of the liquid by dividing the weight of the liquid contained the pycnometer by the weight of water contained, both determined at 25°C.

Determination of viscosity:

The viscosity determinations were carried out using a Brookfield Viscometer (DV II+ Pro model) using spindle number S-63 AT 1 rpm at a temperature of 25° C. The determinations were carried out in triplicate and the average of three readings was recorded.

Determination of Sugar content:5

Clarifying reagent:

Solution I: Dissolve 21.9 g of zinc acetate and 3 ml of glacial acetic acid in purified water and make the volume to 100 ml.

Solution II: Dissolve 10.6 g of potassium ferrocyanide in water and make up to 100 ml.

Reducing sugar: Take suitable amount of the sample and neutralize with sodium hydroxide solution (10 percent in water) evaporate the neutralized solution to half the volume on a water bath at 50°C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II. Mix, filter through a dry filter paper and make up the volume to 100 ml. take 10 ml of the Fehling's solution and from burette and add sugar solution in a drop wise manner and heat to boiling over the hot plate (maintain at 80°C) until the mixture of Fehling's solution appears to be nearly reduced. add 3-5 drops of 1 percent methylene blue and continue the titration till the blue colour is discharged. note down the readings and calculate percentage of sugar.

Non-Reducing sugar:

Take a suitable amount of sample and neutralize with sodium hydroxide solution (10% in water). Evaporate the neutralized solution to half the volume on a water bath at 50°C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II mix, filter through a dry filter paper. To the filter add 15 ml of 0.1 N HCL. Cover the stopper and heat to boiling for two min. add phenolphthalein and neutralize with sodium hydroxide solution (10 %) transfer to 100 ml volumetric flask and make the volume to 100 ml and perform the titration as done for the reducing sugars. Calculate the % of total sugars. Subtract the % of the reducing sugars from the sugars to obtain non-reducing sugars.5

Microbial Analysis:5,7

Sampling: Use 10 g specimens for each of the specified in the individual monograph.

Precaution: The microbial limit should be carried out under conditions designed to avoid accidental contamination during the test. The precaution taken to avoid contamination must be such that they do not adversely affect any micro-organisms that should be revealed in the test

8.1 Total Aerobic Microbial Count:

Water Soluble Product: Dissolve 10 g or dilute 10 ml of the sample preparation being examined, unless otherwise specified, in buffered sodium chloride –peptone solution pH 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the pH to about 7.

8.2 Plate count for Bacteria:

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated sample preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45°C. Alternatively, spread the pretreated

preparation on the surface of the solidified medium in a petri dish of the same diameter. If necessary, dilute the pretreated preparation as describe above so that a colony count of not more than 300 may be expected. prepare at least two such petri dishes using the same dilution and incubate at 30°C to 35°C for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.5

8.3 Plate count for fungi:

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the sample preparation and about 15 ml of liquified casein sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 200C to 250C for 5 days, unless a more reliable count is obtained in a shorter time. Alternatively, spread the pre-treated extract preparation on the surface of the solidified medium in the petri dish of the same diameter. If necessary, dilute the pre-treated sample preparation as describe above so that a colony count of not more than 100 may be expected. Prepare at least two such petri dishes using the same dilution and incubate at 30°C to 35°C for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies are formed. Calculate the results using plates with not more than 100 colonies.

High Performance Thin Layer Chromatography (HPTLC):5,7

Following are the chromatographic conditions required to get an effective resolution selected mobile phase.

Stationaryphase : HPTLC pre-coated, silica gel G 60 F254 (Merck, Germany)

Size : 10 x 10cm

Developing Chamber : Twin trough glass chamber Modeofapplication : Band

Bandsize : 5 mm SeparationTechnique : Ascending Temperature : 25 ± 50C

SaturationTime : 20 min

Scanningmode : Absorbance/Reflection

Preparation of sample solution:

1. Take 15 ml sample of Ashwagandharista-T in iodine flask.
2. Add 50 ml n-butanol for 45 min, cool andfilter.
3. The remaining residue refluxed again with 30 ml n-butanol for 20min.
4. Again cool, filter and combine the washing.
5. Then it was dried on water bath.
6. Dried residue diluted with 5ml of n-butanol in volumetric flask.
7. Repeat the same procedure for Ashwagandharista-M Sample.

Selection of Solvent Phase

Different solvent systems were tried and finally on the basis of best resolution of components, Benzene: Ethyl acetate (9:1) was finalized for HPTLC development of two different phytoconstituent.

Application of Sample

A sample was dissolved in methanol and was applied on pre-coated plate with the help of Linomat IV semi-automatic applicator.

Development of Plate

A rectangular twin trough glass chamber was used in the experiment. To avoid insufficient chamber saturation and the undesirable edge effect, the chamber was allowed to saturate for 20 min before use. The experiment was carried out at room temperature in diffused day light.

Procedure

A pre-activated and sample loaded plate was also dipped simultaneously in chromatographic chamber on another side of the solvent system, allowed to elute upto 8 cm and was air dried. The bands were scanned in CAMAG TLC scanner-3 (Wincat 1.4.1 software)

RESULTS AND DISCUSSION:

Physicochemical parameter:

The Macro-scopical / Organoleptic characteristics of formulation of Ashwagandharista prepared by traditional and modern method are given in table 3 and 4.

Evaluation parameter:

The evaluation parameters include different parameter such as pH, percentage alcohol content, total solid, sugar content,

Table 3. Macro-scopical / Organoleptic characteristics of Ashwagandharista-T.

Description	Day 15	Day 30	Day 45
Colour	Slight cream to Brownish	Slight cream to brownish	Brownish
Taste Bitter	Bitter/ Tasteless	Sour	Bitter/Sour
Odour	Mild Alcoholic Odour	Alcoholic Odour	Alcoholic Odour

Table 4. Macro-scopical / Organoleptic characteristics of Ashwagandharista-M.

Description	Day 15	Day 30	Day 45
Colour	Slight cream to Brownish	Slight cream to brownish	Brownish
Taste Bitter	Unpleasant / Tasteless	Sour	Bitter/Sour
Odour	Unpleasant Mild Alcoholic Odour	Alcoholic Odour	Alcoholic Odour

wt/ml, viscosity specific gravity etc. This parameter is more important for the safety and efficacy of formulation of Ashwagandharista

Determination of pH:

The formulation of Ashwagandharista prepared by traditional and modern method both the sample of Ashwagandharista determined the pH in 3 times (15 days, 30 day, 45 days) the results given in table no.5.

Table 5. Determination of pH

Sample	Day 15	Day 30	Day 45
Sample (T)pH	3.9	4.2	4.2
Sample (M) pH	4.0	4.9	5.1

Determination of Alcohol Percentage:

The formulation of Ashwagandharista prepared by traditional and modern method both the sample of Ashwagandharista determined the alcohol percent in 2 times (15 days, 30 day) the results given in table no.6.

Table 6. Determination of alcohol

Sample	Day 15	Day 30	Day 45
Alcohol content (sample T)	8 %	10.8 %	4 – 10 %
Alcohol content (sample M)	10.1 %	11%	4 – 10 %

Evaluation of Ashwagandharista-T:

The Ashwagandharista prepared by tradition method is evaluated parameters of results given in table no.7.

Table 7. Evaluation of Ashwagandharista-T.

Parameter	Result	Limit
Total solid content	30.08 %	NMT 40 %
Wt. /ml	0.99201 g/ml	-
Viscosity	20 cps	-
Specific gravity at 25°C	0.99597	1 – 1.15
Sugar content	33 %	30-40 %
Microbial count	Nil	NMT 104 CFU/ml

Evaluation of Ashwagandharista-M:

The Ashwagandharista prepared by modern method are evaluated parameters of results given in table no.8.

The physicochemical evaluation of formulation of Ashwagandharista prepared by traditional and modern method are comparatively evaluate and results found to be under limits as well as safe and effective of both the formulation of Ashwagandharista was done.

Table 8. Evaluation of Ashwagandharista-M.

Parameter	Result	Limit
Total solid content	26.49 %	NMT 40 %
Wt. /ml	0.9917 g/ml	-
Viscosity	22 cps	-
Specific gravity at 25°C	0.9956	1 – 1.15
Sugar content	34.33 %	30-40 %
Microbial count	Nil	NMT 104 CFU/ml

High Performance Thin Layer Chromatography:

The HPTLC analysis of the formulation of Ashwagandharista prepared by tradition and modern method are given the results. (fig 1, 2)

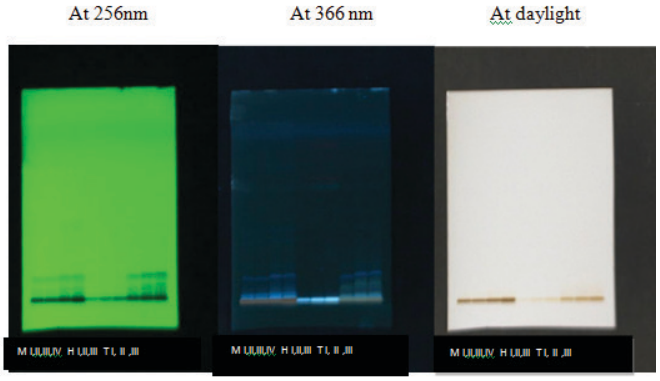

Figure 1: Photo documentation of Ashwagandharista-M and Ashwagandha herb extract and Ashwagandharista-T (Before Derivatization).

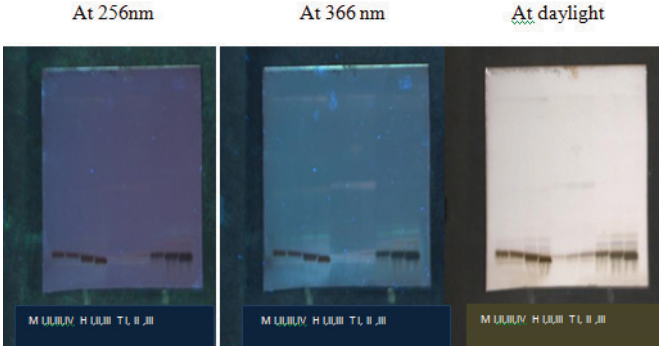

Figure 2: Photo documentation of Ashwagandharista-M and Ashwagandha herb extract and Ashwagandharista-T (AfterDerivatization).

Table 9. Represent of Peak Table.

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Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.76 Rf	2.8 AU	0.78 Rf	59.5 AU	00.00 %	0.79 Rf	0.0 AU	4841.4 AU	00.00 %	AutoGenerated1
5	1	0.75 Rf	7.2 AU	0.77 Rf	15.3 AU	00.00 %	0.79 Rf	0.0 AU	7263.6 AU	00.00 %	AutoGenerated1
6	1	0.76 Rf	9.8 AU	0.78 Rf	96.5 AU	00.00 %	0.79 Rf	0.0 AU	3237.6 AU	00.00 %	AutoGenerated1
7	1	0.75 Rf	7.5 AU	0.78 Rf	53.5 AU	00.00 %	0.79 Rf	2.2 AU	5060.7 AU	00.00 %	AutoGenerated1
8	1	0.73 Rf	5.4 AU	0.76 Rf	17.5 AU	00.00 %	0.79 Rf	1.8 AU	7782.3 AU	00.00 %	AutoGenerated1
9	1	0.74 Rf	8.1 AU	0.77 Rf	82.0 AU	00.00 %	0.79 Rf	7.1 AU	9740.8 AU	00.00 %	AutoGenerated1
10	1	0.74 Rf	7.9 AU	0.76 Rf	73.4 AU	00.00 %	0.78 Rf	7.4 AU	9266.8 AU	00.00 %	AutoGenerated1

Table no.9 shows Rf values of formulation were compared with the Rf values of ashwagandha herb extract and both formulation of Ashwagandharista prepared by traditional and modern method and formulation are similar revealing complete compatibility and Phytoconstituents were found to be intact reveling the stability of formulation.

Peak table:

Indicate the Rf values of formulation of Ashwagandharista prepared by traditional and modern method. (Table 10)

Table 10. Represent of Rf values.

Track	Peak	Start position	Max position
4	1	0.76 Rf	0.78 Rf
5	1	0.75 Rf	0.77 Rf
6	1	0.76 Rf	0.78 Rf
7	1	0.75 Rf	0.78 Rf
8	1	0.73 Rf	0.76 Rf
9	1	0.74 Rf	0.77 Rf
10	1	0.74 Rf	0.76 Rf

Densiogram diagram:

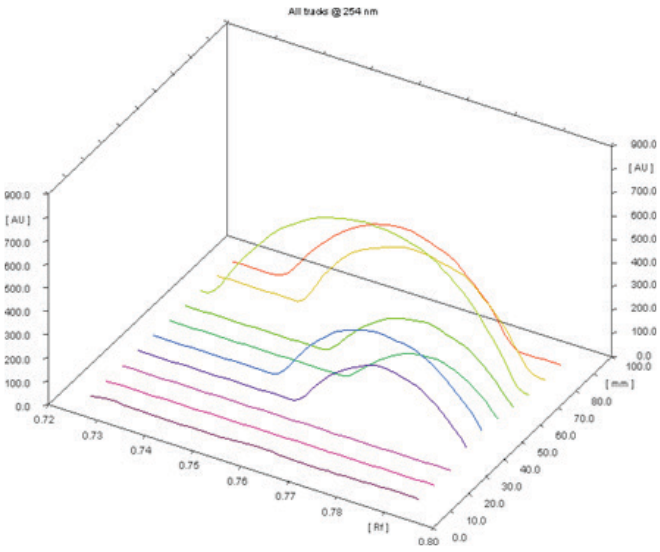

Figure 3: Interpretation of Densiogram diagram.

Spectral Scan Display:

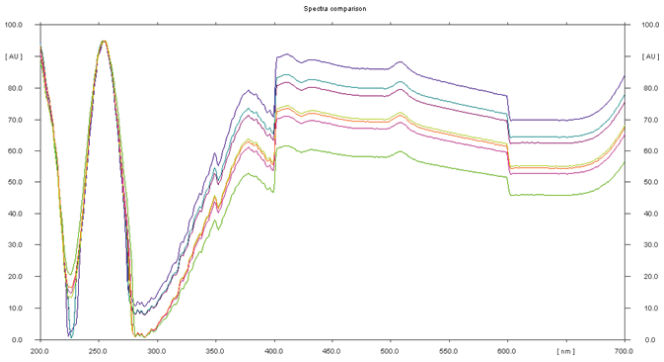

Figure 4: Represent of Spectral Scan Display.

The graph (Fig no.3) of formulations were compared with the peak of ashwagandha herbs extract and formulated Ashwagandharista prepared by traditional and modern method and obtaining results of spectra and peak comparatively studied and evaluation was done. (Fig 4)

CONCLUSION

From this study, we conclude that the time required for formulation of Ashwagandharista prepared by different methods (Traditional & Modern method) can be reduced up to 20 % by modification in the process without affecting quality and efficacy of formulation. In comparative evaluation of Ashwagandharista by traditional and Modern method, the results were found to be under limits and safe as well as effective of both the formulations.

In the study, various physicochemical parameters were evaluated which were found to be within the prescribed limits of WHO guidelines. From the phytochemical evaluations, it was revealed that, the sample of ashwagandha extract showed higher quantity of phytoconstituents as compared to other samples.

In the study, HPTLC analysis shows the Rf values of formulation were compared with the Rf values of ashwagandha herb extract and both formulation of Ashwagandharista prepared by traditional and modern method.

Thus, the study may be helpful in understanding the role of fermenter in the formulations to the variability in phytochemical composition of medicinal plants such as Withania somnifera. This will help in selecting the best quality medicinal plant product for preparation of herbal formulations with best therapeutic value.

Acknowledgement: Author acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those

articles, journals and books from where the literature for this article has been reviewed and discussed."

Source of funding: Nil

Conflict of Interest:

Authors declare there is no conflict of interest.

Authors' Contribution:

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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