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Research Paper

Formulation of A Simplified Process for Extraction of High Concentration of Bioavailable Withanolides from Ashwagandha Root

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ABSTRACT : *Ashwagandha has been used for centuries in Indian traditional medicine. Ashwagandha contains bioactive compounds called withanolides, which are responsible for diverse biological activity of plant, such as anticancer, anti-inflammatory, antimicrobial, immunoregulatory, trypanocidal, and leishmanicidal activity. The present studyaimed to find a simplified process for the isolation of withanolideswith a unique ratio of aglycones to Withanolide glycosides (1:1) from ashwagandha root.The alcoholic extract prepared from the root of Ashwagandha was dried up to 30% solid contents then 2.5 volumes of cow's fat milk was added to prepare the milk extract. The milk extract was mixed with the water extract in a ratio ranging from 1: 1 to 1:10. We invented a simplified process for producing a high concentration (5%) of Withanolides with a unique ratio of aglycones to glycosides (1:1) and negligible content of withaferin A from the root of Ashwagandha. The study also provides a method of improving the bioactivity of Withanolides even at lower doses, with various formulations without using any pharmaceutical excipients, thus enhancing the absorption.*

KEYWORDS: *Ashwagandha, Ashwagandha milk extract, bioabsorption, Withanolides*

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I. INTRODUCTION

Ashwagandha, also known as *Withaniasomnifera*, is an evergreen shrub that belongs to the Solanaceae or nightshade family. It grows in India, the Middle East, and parts of Africa. Ashwagandha has been used for centuries in traditional Indian medicine for its therapeutic properties. It is one of the most extensively researched Ayurvedic medicines[1]. Ashwagandha contains bioactive compounds called withanolides, which are responsible for its therapeutic effects[2]. Withanolides have demonstrated diverse biological activity, such as anticancer, anti-inflammatory, antimicrobial, immunoregulatory, trypanocidal, and leishmanicidal activity. Withanolides are synthesized by *W.somnifera*, which has been extensively investigated in terms of chemistry and bioactivity profiling. In traditional home medicine, Ashwagandha roots have been commonly used for several kinds of herbal formulations, wherein predominant bioactives are Withaferin A, Withanolide A, and Withanone[2,3].

Research has shown that ashwagandha has a rejuvenating and calming influence on the nervous system and, consequently, on the entire being. It has been shown to improve cognitive functions in healthy, stressed adults [4]. A systematic review and meta-analysis found that ashwagandha extract may improve sleep quality. Another systematic review and Bayesian meta-analysis found that ashwagandha may improve physical performance [1]. It is traditionally used as an adaptogen, which is believed to help the body resist physical and mental stress[5].

Ashwagandha extract can be prepared using various methods. Traditional Ayurvedic pharmacopoeias call for the use of ashwagandha root only for internal use, and outline the preferred preparation: a milk extraction process to deliver the full range of bioactives to the body. Modern production techniques can optimize this process[4]. Ashwagandha can also be taken in capsule, gummy, tea, or powder form. The plant

contains a broad spectrum of phytochemicals with a wide range of pharmacological properties [6, 7]. Ashwagandha has been shown to support muscle mass, strength, and recovery. Crude alcoholic extracts of roots and leaves can also be prepared for chemical analysis. *In vitro* propagation and production of withanolides have also been explored using plant tissue culture[8,9].

Withanolide glycosides WS IV and WS V are important neuro-regenerative components found in Ashwagandha. However, these glycosylated and polar withanolide glycosides have low permeability, which reduces their bioavailability. Poor solubility of drug substances is a major challenge in early oral formulation development. When a drug candidate has poor or low solubility, this can have a major impact on the ability of a drug to be absorbed into a patient's gastrointestinal tract[10]. Innovative techniques for solubility enhancement can improve the bioavailability of withanolide glycosides. However, our investigation provides a highly available ashwagandha extract that improves the bioactivity of Withanolides even at lower doses, without using any pharmaceutical excipients, thus enhancing the absorption.

The present study aims to simplify the process of isolating bioactive components in the highest concentration with a unique ratio. The extraction and isolation of natural products have been a bottleneck in the application of natural products in drug development. Therefore, there is an urgent need to develop effective and selective methods for the extraction and isolation of bioactive natural products. The advancement of adequate and selective techniques for the extraction and isolation of bioactive natural products is vital.

II. MATERIALS AND METHODS

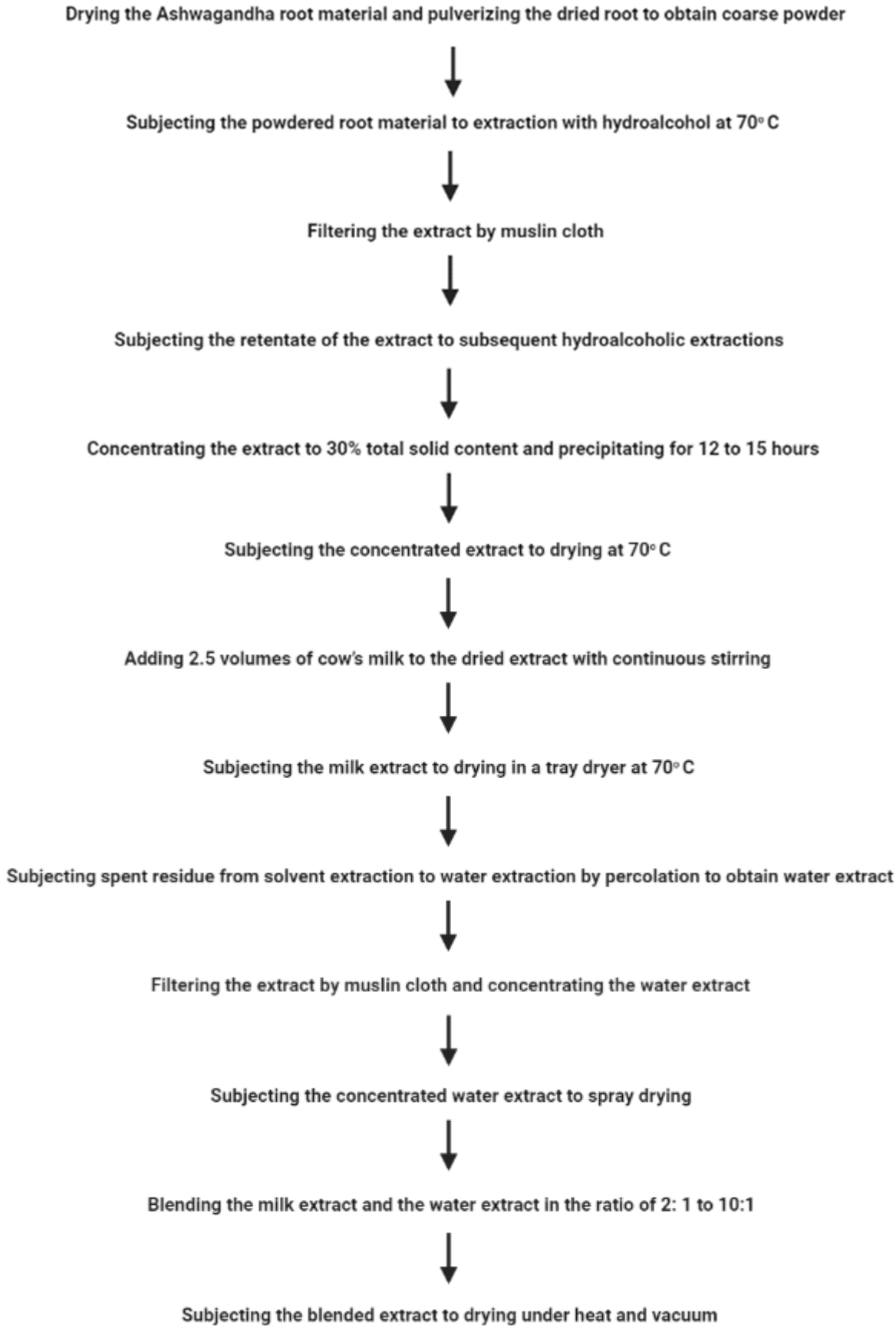
2.1 Processfor isolating the highest concentration of bioactive components from Ashwagandha root

The present study provides a process for preparing Ashwagandha extract rich in Withanolides. The process comprises the following steps. The Ashwagandha root wasdried and pulverised to coarse powder. The powdered root material wasextracted with alcohol, wherein the alcohol was methanol, ethanol, and/or hydro alcohol at 70°C to provide a first extract solution. Filter the first extract solution and repeat at least three times the extraction procedure with the retentate obtained to achieve the complete extract.Concentrate the pooled extract solutions to get an extract containing Withanolides. Strip out the alcohol, concentrate the extract to 30% total solid content, and allow the extract uninterrupted for 12–15 hours to obtain a precipitate settling at the bottom. Dry the precipitate at a temperature not exceeding 70 °C to obtain an Ashwagandha extract rich in Withanolides (5-7% by HPLC) and less than 0.5% withaferin.

2.2 Composition of Ashwagandha extract to enhance the membrane permeability and bioabsorption

The process describes a method for preparing an AshwagandhaComposition, which enhances the bioabsorption and efficacy. In general, this studywas related to Ashwagandha preparation. The current study, in particular, provides a formulation including *W. somnifera* extracts without the use of pharmaceutical excipients and preservatives; this approach includes the following steps (Figure 1).

Fig 1: Process flowchart of highly bioabsorbable ashwagandha Extract



2.3 Preparation of Ashwagandha Milk Extract

Ashwagandha root enriched extract was mixed with 2.5 volumes of cow's fat milk at 60° C for 3–4 hours, stirring constantly to make a homogeneous mass with agitation and concentrate it under a vacuum to a thick paste consistency. Drying the extract at 70 °C in a tray dryer provides the extract with greater bioavailability and enhances the bioactivity.

2.4 Preparation of Ashwagandha Water Extract

To avoid the use of pharmaceutical excipients, Ashwagandha water extract was used in the preparation of herbal solid compositions. Hence, the present disclosure provides a process to make Ashwagandha water extract. Ashwagandha spent (leftover residue after extracting with alcohol) was subjected to extraction with purified water by the percolation method at room temperature. The water extractions after 12–24 hours were filtered through muslin cloth and concentrated into a thick paste. After achieving the desired total solid content, the soft extract was spray dried to a free-flowing dry extract powder. The water extract was also prepared by the hot soxhalation method. The obtained water extract was used for the preparation of an AshwagandhaComposition in a stable, highly bioavailable, and non-hygroscopic form.

2.5 Blending

Ashwagandha aqueous extract was suspended in water to which the Ashwagandha milk extract wasaddedto form a mixture. Homogenize the mixture to obtain a fine slurry and dry the fine slurry under heat and vacuum to form a uniform blend of the composition having the Ashwagandha milk extract and aqueous extract.

In the present studythe processprovides a solid formulation that comprises a blend of Ashwagandha milk extract and water extract of Ashwagandha, wherein the said blend of extract and said powder of water extract are mixed in a ratio of about 1: 0.5 to 10: 1.

2.6 Method of preparing a tablet containing a composition having Ashwagandha milk extract and Ashwagandha aqueous extract without the use of pharmaceutical excipients and preservatives

The process of preparing the Ashwagandha solid formulation involves granulation of the blend of extracts using a solvent system of ethanol for granulating the mixture and autoclaving the granules. Autoclaving helps in microbial control of the solid formulation as it does not contain any preservatives.

The autoclaved granules were further compressed or encapsulated into tablets or capsules. The Ashwagandha milk extract and the aqueous extract of the herb were mixed in a predetermined ratio, preferably between about 1: 1 and about 1:10 for optimum granulation.

All extracts, granules, and tablets were subjected to standardization by High Performance Liquid Chromatography (HPLC) for quantitative estimation of active marker compounds. The solid formulation according to the present study has a desired hardness of preferably between about 3 and about 4 kg/cm, friability of less than about 1%, and a disintegration time of less than about 30 min. Here, the solid formulation waspreferably granules, tablets, or capsules.

2.7 Granulation and Compression Manufacturing Procedures

2.7.1 Dispensing and Shifting of extracts:

The extracts were dispensed into batches and checked forloss on drying (LOD), bulk density (BD), and Microbiological Analysis.Ashwagandha water extract sifted through a #40 sieve.The above-sifted materials were collected in separate duly labelled double-lined polybags. The amount sifted as well as the sieve integrity before and after sifting were recorded.

2.7.2 Dispensing and Shifting of extracts:

Charged 50 kg of aqueous extract and 50 kg of Ashwagandha milk extract were mixed into the rapid mixer granulator (RMG) for about 5 minutes. Ethanol was added to the RMG containing the aqueous extract and Ashwagandha milk extract and mixed over a period of about 3 minutes at a medium speed. The mixer was Stopped and scraped off the mass from the sides and bottom. Mixing was continued by operating the impeller at high speed with the chopper on for about 2 minutes. Additional quantity of ethanol was added if required. Discharged the mass from the RMG.

2.7.3 Wet Milling:

Milled the wet mass obtained in a multi-mill fitted with an 8 mm screen.

2.7.4 Drying:
The wet mass was dried in Tray Drier/ fluidized bed dryer at 55° C to 65° C for about 60 minutes.The moisture was checked once every 30 minutes; (LOD Limit: Not more than 5% w/w) and recorded the details.

2.7.5 Sizing:
The dried granules were sifted using a sifter fitted with a #20 sieve, and the sifted granules were carefully collected in a clean, double-poly-lined HDPE container. The retains (oversize granules) obtained were milled through a Multi Mill fitted with a 1.5 mm screen and knives in the forward direction. The milled granules were then passed through a sifter fitted with a #20 sieve, and the sized granules obtained were collected and added to the previously sifted granules.

The combined granules were blended for about 3 minutes at 20-25 RPM. After blending, the mixture was unloaded into clean double poly-lined HDPE drums, and status labels were duly affixed. The blend was carefully weighed, and all relevant parameters were entered into the records.

To ensure proper manufacturing, the machine was adjusted according to the tooling specifications listed in Table 1.

Table 1: Input parameters

PARAMETER	STANDARD VALUE
Loss on drying	NMT 10% w/w
Bulk density	0.55-0.75 g/ml
Granules to fine ratio	60:40-90:10
Actives	Total Withanolides by HPLC, NLT 2.5% as per USP
TAMC	NMT5000 CFU/gm
Fungal count	NMT 100 CFU/gm

III. RESULTS AND DISCUSSION

3.1 Isolating the highest concentration of bioactive components from Ashwagandha root.
A simplified process for producing a high concentration (5%) of Withanolides with a unique ratio of aglycones to glycosides (1:1) and negligible content of withaferin A (0.02%) from the root of Ashwagandhawas described. The present disclosure comprises the steps of subjecting Ashwagandha root to solvent extraction, concentrating said extract, and crystallizing concentrated extract to obtain a high concentration of Withanolides with a negligible amount of withaferin A.

3.2 Improved Ashwagandha extract with enhanced membrane permeability and bioabsorption

The low permeability of the glycosylated and polar withanolide glycosides WS IV and WS V reduces the bioavailability and efficacy of active compounds withanolide glycosides [10]. Therefore, the present study relates to making Ashwagandha more bioavailable to the body, and it uses a purification process where the enriched Ashwagandha extract was steamed in the form of cow's milk and then dried. Thus, the extract was more bioavailable and enhances the bioactivity.

The study provides a composition of Ashwagandha milk extract with a high concentration of Withanolides, wherein the milk extract was present in an amount sufficient to cause an enhancement of membrane permeability and absorption of the Ashwagandha extract when the composition was administered as compared to the bioavailability of the basic Ashwagandha extract obtained upon administration of a composition of Ashwagandha extract that was prepared without adding milk extract. Hence, this study provides a composition of Ashwagandha milk extract with a high concentration of Withanolides, wherein the enhancement of membrane permeability and bioavailability of the Withanolides ranges from about 5-fold to about 15-fold.

3.3 Preparation of tablet containing a composition having Ashwagandha milk extract and Ashwagandha aqueous extract without the use of pharmaceutical excipients and preservatives.

This study provides an herbal solid formulation essentially free of excipients, additives, or preservatives, wherein said formulation comprises a blend of Ashwagandha milk extract, water extract of *W. somnifera*, and a process for preparing the same. The process of preparing the herbal solid formulation involves

granulation of the blend of extracts using a solvent system of ethanol for granulating the mixture and autoclaving the granules. Autoclaving helps in microbial control of the solid formulation as it does not contain any preservatives [11].

3.4 Finished Product Specification of Ashwagandha per tablet

The theoretical average weight of the tablet was 550mg, with a weight uniformity requirement of 550mg ± 5%, indicating an acceptable weight range of 522.5mg to 577.5mg. The weight of 20 tablets had to be within 11.00 g ± 5%, with an acceptable weight range of 10.45 g to 11.55 g. The tablet thickness needed to be between 4.8 mm and 5.58 mm, and the tablet hardness was required to be in the range of 2 to 6 Kg/cm. The friability of the tablet was not supposed to exceed 1.0% WW, and the disintegration time was expected to be no more than 30 minutes. The total withanolide content of the tablet was set to fall between 13.75mg and 15mg. These specifications ensured that the tablet met high-quality standards and was consistent with its intended use.

IV. CONCLUSION

In conclusion, the development of a simplified process for the isolation of bioactive components from Ashwagandha root, with a unique ratio of aglycones to Withanolide glycosides, and without the use of leaves for extraction, offers a promising approach for the production of stable compositions with improved bioactivity. The unique ratio of bioactive components in the extract may have significant applications in various fields such as pharmaceuticals, nutraceuticals, and functional foods. The method of improving the bioactivity of Withanolides at lower doses, without using any pharmaceutical excipients, may offer an alternative approach to conventional pharmaceutical formulations, which may have fewer side effects and improved absorption. Further studies and research are required to explore the potential benefits and applications of this innovative extraction method.

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