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Review Article

A new approach phytochemicals analysis and exploration of therapeutic applications of shatavari, ashwagandha roots

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Abstract

Withania somnifera and asparagus racemosus are very important medicinal plants, climbing shrubs from the important Ayurveda system of medicine, which acts as one of the main sources of new pharmaceutical and health care products. It is commonly deployed as a boost for immunity, anti-inflammatory, and anti-stress and is found throughout India. The medicinal qualities and helpful uses of both, as well as their phytochemicals, are important as valuable medicinal plants. This paper presents research on the phytochemical analysis of new approaches using shatavari and ashwagandha roots. It is also increasing the life of humans by preventing from many chronic diseases. The shatavari and ashwagandha roots are well known for their immunity-boosting applications and prescribed for the treatments of chronic high temperature, swine flu, malaria, stress, diabetes, skin problems, urinary problems, etc. We are preparing new approaches to health benefits using the chemical substance of some of these herbals belongs to different classes, such as saponins, alkaloids, steroids, flavonoids, glycosides, and polysaccharide-like compounds having medicinal properties.

Introduction

Ashwaganddha root

Ashwagandha is the ayurvedic herbal plant. It is also used for chronic disease enhancement, for example, neurologist disease, cardiologist, and reproductive disease overcome. It belongs to the Solanaceae family. The Ayurvedic herbal medicine system also makes use of it. Northern and central India are prime locations for its cultivation. The Indian states of Madhya Pradesh, Uttar Pradesh, Gujarat, Haryana, Maharashtra, Punjab, and Rajasthan are the most important regions for the production of Ashwagandha. China, Yemen, and Nepal are also cultivators of this plant.

Taxonomy

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Solanales

Family: Solanaceae

Genus: Withania

Species: W. somnifera

Figure 1 Ashwagandha roots

Ashwagandha is cultivated in the required climate condition at an altitude of 1500 meters above sea level. It is the cultivated maximum rainfall above the 500–800. It is required that the optimum temperature be 20–38°C for growing the dry plant. The herbs are used for their beneficial effects in a wide range of diseases, including Parkinson's disease, neurological disorders, Alzheimer's disease, and reproductive diseases. It is also known by the English name winter cherry. It is the Indian name of Ashwagandha, Varahakarni (Sanskrit). Askandhatilli, Punir (Hindi).

Properties

Medicinal properties:- Ashwagandha have multiple medicinal properties those are used traditionally medicine as well as 21s century's people are also preferred for better health.

- Stress reduces
- Improve sleep

- Enhanced cognitive function
- Increase energy and vitality
- Anti-inflammatory effect
- Hormonal balance
- Anti-oxidant properties

Withanalide

There is a special family of steroidal lactones known as withanolides. Within the Solanaceae family, fifteen genera include more than one hundred thirty withanolides. Some marine organisms, other groups of Leguminosae and Labiatae, and other plant families include withanolides [1-5]. The withanolides with a highly functional group are most abundant in W. Somnifera plants. The primary biochemicals produced by W. Somnifera, withanolides, are mostly found in the plant's leaves and roots and have a dry weight ranging from 0.001% to 0.5% [2-4]. Natural steroidal lactones with 28 carbon atoms are generated by oxidizing C-22 and C-26 to six- or five-membered lactone rings on an ergostane skeleton. These rings are then used to form withanolides. The 22-hydroxy ergostane-26-oic acid 26, 22-lactones, which make up the basic structure, are called the withanolide skeleton [3-9]. Both withanolide A and withanolide B are able to block amyloid beta, the protein responsible for Alzheimer's disease; however, withanolide A differs from withanolide B due to the inclusion of an extra hydroxyl group on the C-20 atom.[4]

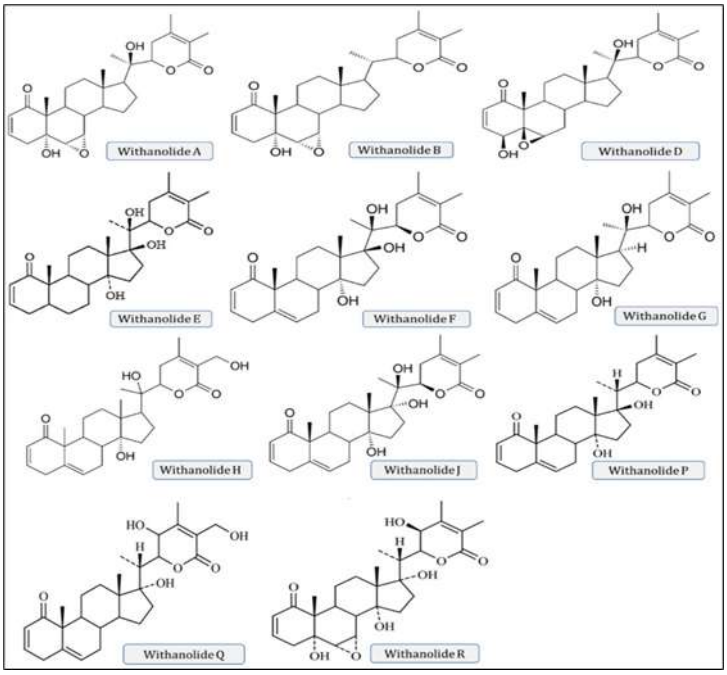


Figure 2 Withanalides chemical structure

Withaferin A

4β,27-dihydroxy-1-oxo-5β, 6β-epoxywitha-2-24-dienolide, also known as withaferin A, it was first isolated from in W. somnifera leaves of a South Asian variety at concentration of 0.13–0.31% dry weight. The quantitative analysis of

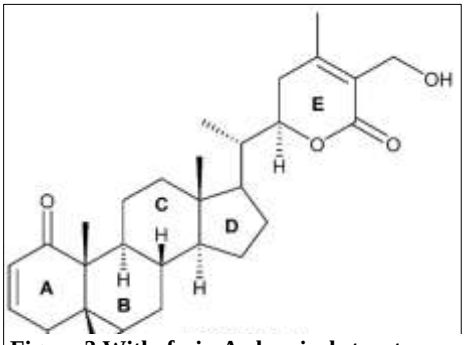


Figure 3 Withaferin A chemical structure

W. somnifera by TLC and HPLC analysis showed that withaferin A compound was present at of 1.6% in leaves, while it is present at very low concentrations in roots and [5-10].

Withanamide

A methanolic extract of the roots of Withanamides somnifera is used for purification. Only in the fatty acid side chain does withanamide A differ structurally from withanamide C; in contrast, withanamide A contains two double-bonds in its side chain. The roles of withanamide A and withanamide C are to protect cells from cell death and to counteract the harmful effects of beta-amyloid protein (BAP). One chemical that has shown promise in the treatment of Alzheimer's disease is Withanamide A [43]. Compared to serotonin, withanamides are superior antioxidants and have the same level of activity against lipid peroxidation (LPO) as commercially available antioxidants such as BHA, BHT, and TBHQ. The hydroxylated long-chain acyl group is likely responsible for their antioxidant action [10-13].

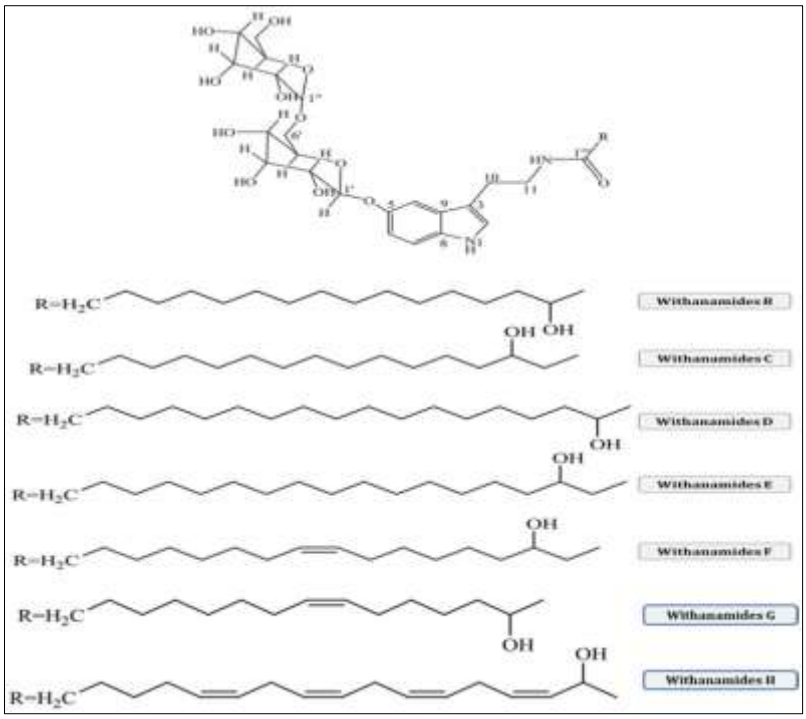


Figure 4 Withanamide chemical structure

Withanoside

One class of compounds produced by W. Somnifera is called withanolides glycosides or glycowithanolide. Glycosidic connections often consist of 6O-ß-D-glucopyranosyl-ß-D-glupyranisyl compounds. The roots of W. Somnifera were found to contain seven different withanolide glycosides, which are numbered I through VII [13-15]. In contrast to withanolide, withanosides have an additional hydroxyl group on the carbon atoms 20 and 27. Withanoside IV differs from withanoside V, while sharing the same structural formula, because it has an extra hydroxyl group attached to the C-27 atom. Research has demonstrated that withanosides IV and V prevent the creation of Alzheimer's disease-causing amyloid ß peptide fibrils [46,47], but withanosides IV and VI enhance dendritic formation [15-18].

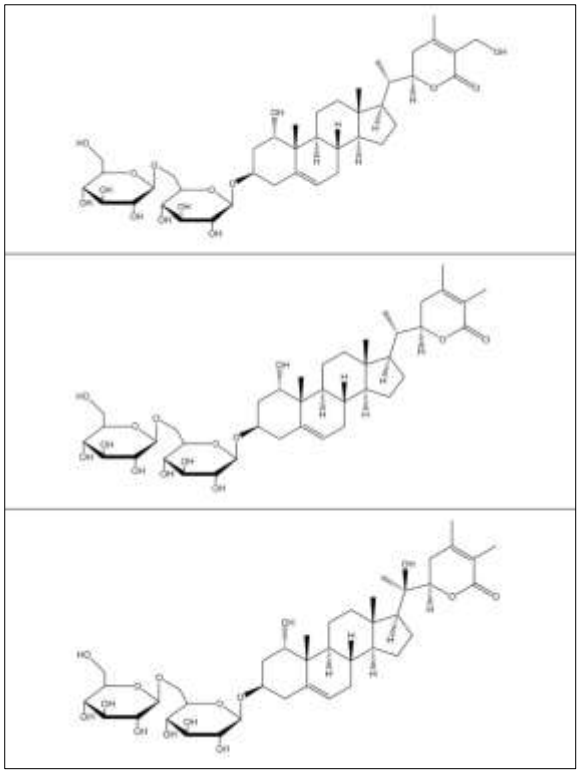


Figure 5 Withanosides chemical structures

Shatavari root

The genus Asparagus has over 300 species worldwide, 22 of which were reported in India.

A. Racemosus is extensively spread around the globe and its distribution varies from tropical Africa, Java, Australia, Sri Lanka, southern portions of China, and India, but it is primarily grown in India[18-25].

Taxonomy

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Asparagaceae

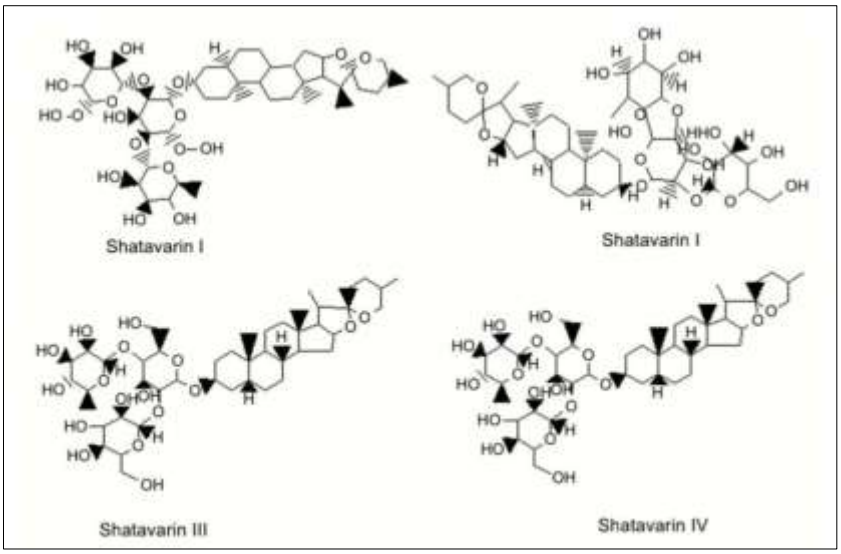
Subfamily: Asparagolite

Genus: Asparagus

Species: A. Racemosus

Figure 6 Shatavari roots

A. racemosus is an important medicinal plant which is regarded as a 'rasayana' which means plant drugs promoting general well-being by increasing cellular vitality and resistance · Use of A. racemosus is mentioned in the ancient literature of Ayurveda (Charaka samhita). Traditionally, A. racemosus is indicated in epilepsy, vata disorders , brain tonic, helps in regulating cardiac disorders and hypertension [18] .



Asparagus racemosus roots is very beneficial for woman health. To enhance fertility and hormonal balance. Alleviates symptoms of menopause, breast, milk production. These are also beneficial for man health improves sperm quality and overall reproductive health. As ayurvedic we always used traditionally, because very helpful in digestion problem, immune system, hormonal balance, stress and anxiety management[25-30].

PROPERTIES

Racemoside A,B and C

This compound is extracted from the root of asparagus racemosus, and these are a steroidal saponin. It shows as anti-leishmanial activity. Racemoside A may be effective against both drug-sensitive and drug-resistant strains of Leishmania parasites. But these are not approved; they are under research on-going clinical studies[34-40].

Racemoside B and C are process in research

Shatavari is medicinal plant their various uses in human body as medicine. It has multiples properties those are use in medical field.

Anti-Oxidant

Anti-inflammatory

Hormone imbalance

Anti-depressant activity

Anti-anxiety effect

Phytochemical contents

The phytochemical screening of extracts of shatavari root showed the presence of carbohydrate, flavonoids, alkaloids, phenolic compound and tannins in ethanolic as well as aqueous extract while, steroids and/or terpenes and saponin in ethanolic extract. Shatavari also constituents' essential oils, vitamins, asparagine, arginine, tyrosine, flavonoids, resin, and tannins as some other primary chemical constituent is shatavarin I-IV. [30-3

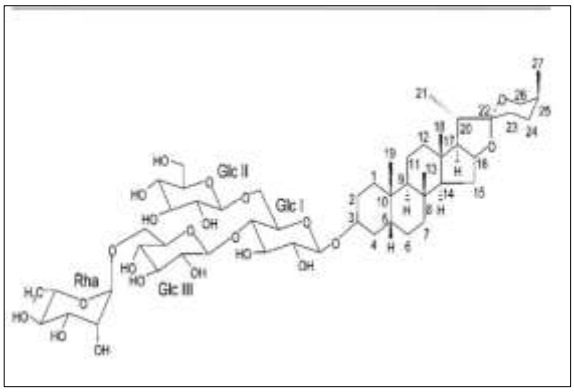


Figure 7 : Racemoside A chemical structure

Asparagamine A

It is an alkaloid, and also call as Didehydrostemofoline. Those are using anti-neoplastic, anti-oxidant, and anti-inflammatory[40-42].

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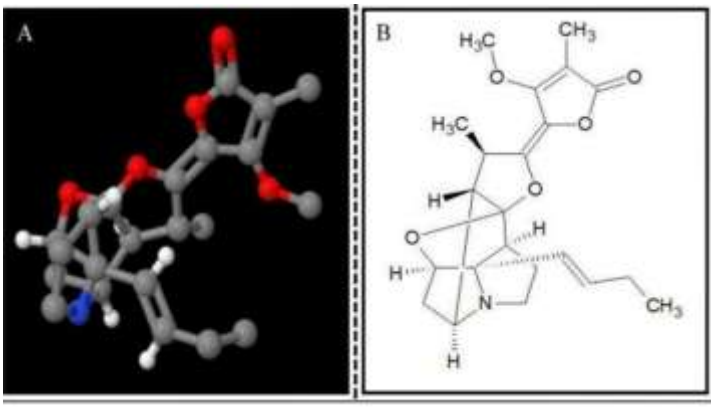

Asparanin A

Chemical Class: Steroidal saponin, Molecular Formula: $C_{39}H_{62}O_{14}$, Molecular Weight: 738.9 g/mol, Structure: Contains a spirostanol skeleton with sugar moieties. On this compound keep going research and scientist find that is cancelled be used as anti-cancer, manage oxidative stress and boost immunity. [42-45].

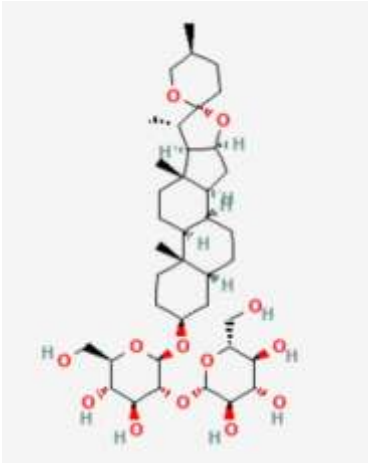

Figure 8 Asparanin A chemical structure

Racemosol

Racemosol is a bioactive compound isolated from root of asparagus racemosus; a plant renowned for it medicinal properties in traditional medicine. Chemically Racemosol is identified as 5-methoxy-13,16,16- trimethyl-15-oxatetracyclo[8.7.1.0^{2},^{7}.0^{14},^{18}]octadeca-2(7),3,5,10,12,14(18)-hexene-6,12-diol, with the molecular formula C21H24O4

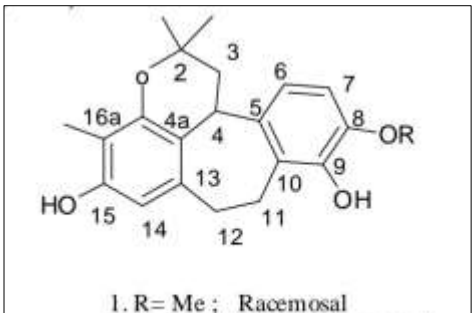

Figure 9 Racemosol chemical structure

Asparagine

Asparagine is an amino acid that plays a crucial role in protein synthesis and metabolism. While it is not typically considered a “bioactive” compound in the way that plant secondary metabolites or drugs are, it does have important physiological roles. Below are some key aspects of its chemical bioactivity. That is uses to maintain the equilibrium in central nervous system, those are non-essential amino acid in our body[45-50].

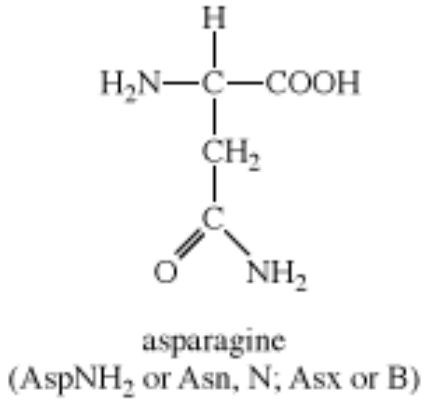

Figure 10 Asparagine chemical structure

Table 1: Phytochemical bioactive construct of Shatavari.

Category	**Isolated compound**	**Activity**
Steroidal Saponins	Shatavarin I-IV Racemoside A Racemoside B Racemoside C Asparanin A	Anti-oxidant Anti-inflammatory
Alkaloid	Asparagamine A Polycyclic alkaloid	Anti-oxidant, Immunomodulator, Ant abortifacient
Flavonoid	Kaempferol Quercetin Hyperoside Quercetin-3-glucuronide	Anti-oxidant, Anti-microbial, Cardioprotective Anti- cancer
Fatty acids	Thymol Eugenol Menthol	Anti-microbial
Minerals	Ca, Cu, Fe, K, Zn etc.	
vitamins	A, B1, B2, C, E, and K etc.	

Method And Material

Plant Material Collection

Roots of Asparagus racemosus and Withania somnifera were collected from authenticated sources and identified by botanical experts.

Extraction Methods: Solvent extraction was performed using ethanol, methanol, and water to obtain bioactive compounds.

Extraction of a Ashwagandha roots :-
Infusion is a straightforward method for extracting Ashwagandha roots. The ashwagandha extraction method can include the following steps:

- Firstly, remove the dust and contamination from the roots.
- Cut the roots in smalls pieces.
- After that crushed the roots and reduced the particle size and converted into powder form.
- Then weighted of 20-gram *ashwagandha* with the help of weighing balance.
- *Ashwagandha* mixed with 40 ml water and 40 ml methanol.
- Then keep on water bath at the temperature of 60^0 C for 4-5 hour.
- After this process left it to cool for 15 minutes.
- Now filtered solution of *ashwagandha* and collected it.
- After keep on water bath for the evaporation of filtered part.
- Now repeat the process until the colour change of the solution.
- Then we will burn all the water that is left in it and collected the extraction in sticky form. [50-55].

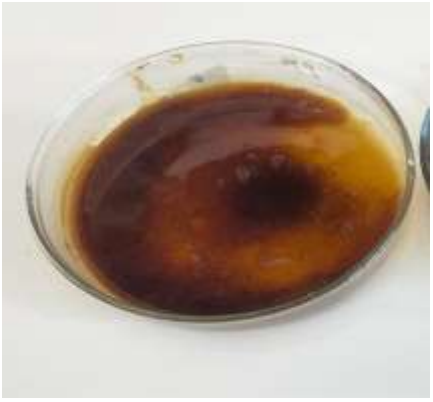

Figure 11 Ashwagandha root extract

Characterization of ashwaganddha

. Carbohydrates test:-

Molisch's test

- Added 2ml of distilled water and added 2 drops of molisch reagent to each other test tube.
- Hold the test tube at incline and carefully added 1ml of concentrated sulphuric acid down the side of test tube.
- A purple or violet ring indicates the presence of carbohydrates. [55-60].

Figure 12 Molisch's test

Alkaloid test

Mayer test

- First of all, we take 2ml concentrated HCl .
- Then 2ml added extract/sample.
- Then added a few drops of mayer's reagents.
- A white precipitate or green colour indicate the presence of alkaloids.

Dragendorff's test

- Then indicate bright yellow precipitate formed.
- Added 1ml of dragendorff's reagent to 2ml of extract.
- After that orange or orange-red precipitate formed.
- Alkaloids are present.

Hagar's test

- First of all, a few drops of Hagar's reagents (saturated picric acid solution).
- Added to 2ml of extract.

Figure 13 Mayer's , Dragendorff's , Hagar's test of ashwagandha root

Flavonoid test

Alkaline Reagent Test

- Add 2 mL of the extract to 2 mL of 2% sodium hydroxide solution
- Observe the color change.
- Add a few drops of dilute hydrochloric acid.

Observation

A yellow color that disappears after the addition of HCl confirms the presence of flavonoids.

Starch test:-

Iodine Test for Starch

- Take 2–3 mL of the extract in a test tube.
- Add 2–3 drops of iodine solution to the extract. [60-65].
- Mix gently and observe the color change.

Observation

The appearance of a blue-black color indicates the presence of starch.

If no starch is present, the solution remains yellowish-brown (color of iodine).

Figure 14 Iodine test

Glycoside

Keller-Kilani test

- A mixture of glacial acetic acid, FeCl3, and conc. H2SO4 is added to an extract.
- If cardiac glycoside is present, a brown ring was form between the layers.

Extraction of shatavari roots

shatawari is an herb used in Ayurveda medicine. The extraction process for *shatawari* can involve the following steps

- First of all, remove the dust and contamination from the roots.
- Cut the roots in smalls pieces
- After that crushed the roots and reduced the particle size and converted into powder form. [65-68].
- Then weighted of 20-gram *shatawari* with the help of weighing balance.
- *shatawari* mixed with 40 ml water and 40 ml methanol.
- Then keep on water bath at the temperature of 60^0 C for 4-5 hour.
- After this process left it to cool for 15 minutes.
- Now filtered solution of *shatawari* and collected it.
- After keep on water bath for the evaporation of filtered part.
- Now repeat the process until the colour change of the solution.
- Then we will burn all the water that is left in it and collected the extraction in sticky form. [68-71].

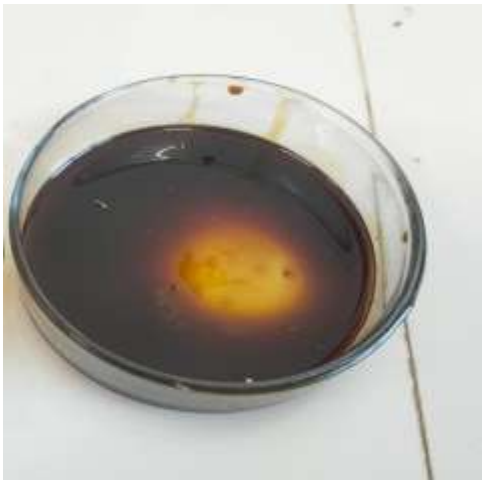

Figure 15 Extraction of Shatavari

Characterization of shatavari

Carbohydrates test

1. Molisch's test

- Added 2ml of distilled water and added 2 drops of molisch reagent to each other test tube.

:

- Hold the test tube at incline and carefully added 1ml of concentrated sulphuric acid down the side of test tube.
- A purple or violet ring indicates the presence of carbohydrates. [71-77].

Figure 16 Molisch's test of Shatavari

Alkaloid test:

Mayer test

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- Then added a few drops of mayer's reagents.
- A white precipitate or green colour indicate the presence of alkaloids.

Dragendorff's test

- Added 1ml of dragendorff's reagent to 2ml of extract.
- After that orange or orange-red precipitate formed.
- Alkaloids are present.

Hagar's test

- First of all, a few drops of Hagar's reagents (saturated picric acid solution).
- Added to 2ml of extract.

- Then indicate bright yellow precipitate formed [15-18].

Figure 17 Mayer's , Dragendorff's , Hagar's test of Shatavari root

Flavonoid test

1. **Alkaline Reagent Test:**

- Add 2 mL of the extract to 2 mL of 2% sodium hydroxide solution.
- Observe the color change.
- Add a few drops of dilute hydrochloric acid.

Observation

A yellow color that disappears after the addition of HCl confirms the presence of flavonoids.

Starch test

1. **Iodine Test for Starch**

- Take 2–3 mL of the extract in a test tube.
- Add 2–3 drops of iodine solution to the extract.
- Mix gently and observe the color change.

Observation

The appearance of a blue-black color indicates the presence of starch.

If no starch is present, the solution remains yellowish-brown (color of iodine).

Figure 18 Iodine test

In-vitro and In-vivo Studies

Antioxidant Activity: Assessed using DPPH and ABTS radical scavenging assays.

Adaptogenic Effect: Evaluated through in-vivo stress-induced models in mice.

Anti-inflammatory Activity: Investigated using carrageenan-induced paw edema in rats.

Discussion

Synergistic effects :- Withania somnifera are given anti- cataleptic effect of could be attributed to its polyphenols, which are responsible for direct free radicals, and the inhibition of lipid peroxidation in the central nervous system. Both herbs are adaptogenic, which may enhance stress tolerance, reduce cortisol levels, and support overall homeostasis. If they are showing synergistic effects that can be so they are boost immunity, hormonal balance, reduce stress levels, improve vitality in human body. Both are can be increasing antioxidant activity through withanolides, flavonoids and saponines etc.

Antagonist effects :- Shatavari has estrogenic effects, which could interfere with Ashwagandha's balancing effects on hormones, especially in individuals with hormonal disorders. If the combination over-stimulates certain pathways, they could counteract or reduce each other's effects, such as in specific stress or immune conditions.

Pharmacokinetic interactions

Both Shatavari and Ashwagandha roots contain several bioactive compounds. If they will have pharmacokinetic interactions, that interactions will produce two responses positive or negative response. For example, if we assume that it will produce a positive response, it will undoubtedly benefit health by reducing stress, increasing energy and vitality, hormonal balance, and improving the reproductive system[7o]. Shatavari contains asparagine amino acids, which can be beneficial to human mind and health and their white setup. For example, if any human asparagine amino acid is unable to proper synthesis that in case we can use asparagine amino acids.

Potential cytotoxicity This can cause cytotoxicity if a high dose of withaferin A is given. Care must also be taken that one compound does not interact with another compound and cause toxicity. If there is drug overlapping, this can also lead to cytotoxicity and poor health. And this is the most common thing in making any medicine or formulation.

Expected Result

Therapeutic outcome Both have almost same therapeutic bioactivity so; most chances is that they give synergistic effects. Improve stress response and energy level due to complimentary Adaptogenic action.

Enhance neuro-protective and cognitive support.

It can be given better hormones regulation, potentially benefits of reproductive health in both male and female.

These drugs combining with can be increase strength immunity and reduce inflammation.

Adverse drug reactions

High concentration of saponine can be cause irritation and disturb GIT. Possible hypersensitivity to either plant.

Over stimulation of immune system may be lead auto-immune.

These are in future may be give very effective medicine for human body health. Because they are ayurveda medicine and can make this different formulation .

Conclusion

The combined formulation of both can provide health benefits and synergistic effects, especially in stress management, immune modulation, reproductive health. Because we have been using these for reproductive and immunity purposes since centuries. However, it is important to keep in mind that dosage optimization and safety evaluation is very

important, to avoid toxicity and ADR. Powders of both can be taken with milk or water, this will improve health. In future, some formulations can be made by purifying both of them for health benefits.

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Ethical approval : NA

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Conflict of Interest

The authors assert that they possess no identifiable competing financial interests or personal ties that may have seemingly influenced the work presented in this study. The authors assert the absence of any conflict of interest among themselves. The writers bear sole responsibility for the content and composition of this article.

Financial interests: The authors declare they have no financial interests.

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