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Research Article

# ANTIOXIDANT STUDIES OF ONE AYURVEDIC MEDICINE ASWAGANDHARISHTAM

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**ABSTRACT**

**Objective:** The present study deals with the antioxidant study of one Ayurvedic medicine Aswagandhrishtam by three different methods, namely reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.

**Methods:** Aswagandharishtam, which is a liquid medicine, was taken as such at various concentrations for all the three assays.

**Results:** The results show that Aswagandharistam has good antioxidant potential when compared with ascorbic acid as standard. The $IC_{50}$ values of reducing power assay were 250.142, that of DPPH were 103.607, and of ABTS assay were 197.79 as compared with that of ascorbic acid being 19.59.

**Conclusion:** All the three assays indicated that Aswagandharishtam showed very good antioxidant results.

**Keywords:** Aswagandharishtam, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, 2, 2-Diphenyl-1-picrylhydrazyl, Vata, Antioxidant, Epilepsy, Ascorbic acid.

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## INTRODUCTION

The evaluation of the scientific efficacy of Ayurvedic and Siddha drugs has become an urgent necessity for their acceptance and promotion. Although these systems are in vogue since ages, the scientific validation with modern parameters will prove their efficacy and help in resurrecting these age-old systems of medicine. This is all the more required due to the development of multidrug-resistant pathogens and increase in the incidence of dreaded diseases such as cancer, malaria, and AIDS, with which the modern medicine is unable to cope up with. It will be wise to develop a safe, cost-effective medicine which could have less or no side effects. For the past two decades, increasing focus is being given by government and private players in this direction [1-30]. Ministry of AYUSH, Government of India, and other such organizations should come forth to develop techniques, protocols, and methods to establish the Ayurvedic and Siddha medicines at the global level.

The present study is one step in this direction. The study deals with the antioxidant study of Ashwagandharishtam or Aswagandharishtam, which is a liquid Ayurvedic medicine used in psychiatric conditions, dullness, loss of memory, sluggishness, epilepsy, low digestion power, piles, and diseases caused by Vata imbalance. It is also used as nerve tonic, for sexual disorders and for depression. Being an Arishta, it contains about 5–10 % of self-generated natural alcohol which helps in the delivery of the drug in the body. The dosage of this medicine is usually 12–24 ml twice daily after food or as advised by the physician.

The present work undertakes the antioxidant assay study of this medicine to understand its possible mechanism of action. The manufactures of this medicine are Baidyanath, Dabur, AVN, AVP, Vaidik Herbs, and Kottakkal Arya Vaidya Sala.

Since this medicine helps in rejuvenation of mind and body, its role as an antioxidant should be understood. The present study encompasses three antioxidant assays, namely reducing power assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and 2,2'-azino-bis(3ethylbenzothiazolin6-sulfonic acid (ABTS) assay.

## METHODS

This Arishtam is made of the following ingredients which are divided into two sections: Kwatha dravyas (Main components) and Prakshepa dravyas (additive components).

The coarse powder of Kwatha dravyas (Main components) is added with water, boiled and reduced to 12.288 L, and filtered. It is added with honey, and Prakshepa Dravya (additive components) powders are added and kept in an airtight container for 1 month for fermentation. After a month time, it is filtered and preserved.

Aswagandharishtam ingredients:
Kwatha dravyas (main components)
Ashwagandha (*Withania somnifera*) - Root - 2.4 kg
Mushali (*Chlorophytum tuberosum*) - Root - 960 g
Manjishta (*Rubia cordifolia*) - Root - 480 g
Haritaki (*Terminalia chebula*) - Fruit - 480 g
Nisha - Turmeric - (*Curcuma longa*) rhizome - 480 g
Daruharidra (*Berberis aristata*) - Stem - 480 g
Yashtimadhu - Licorice - (*Glycyrrhiza glabra*) Root - 480 g
Rasna (*Pluchea lanceolata*) - Root/leaf - 480 g
Vidari (*Pueraria tuberosa*) - Root - 480 g
Arjuna (*Terminalia arjuna*) - stem bark - 480 g
Mustaka (*Cyperus rotundus*) - Rhizome - 480 g
Trivrit (*Ipomoea turpethum*) - Root - 480 g
Sariva (Indian sarsaparilla - *Hemidesmus indicus*) - Root - 384 g
Krishna Sariva (*Cryptolepis buchanani*) - Root - 384 g
Shweta Candana (*Santalum album*) - heartwood - 384 g
Rakta Candana (*Pterocarpus santalinus*) - heartwood - 384 g
Vacha (*Acorus calamus*) - Rhizome - 384 g
Chitraka (*Plumbago zeylanica*) - Root - 384 g
Water for decoction - 98.304 L
Boiled and reduced to 12.288 L
Madhu - Honey - 14.4 kg
Prakshepa - Dravyas (additive components)
Dhataki - *Woodfordia fruticosa* - Flower - 768 g

Shunti - Ginger - (*Zingiber officinale*) - Rhizome - 96 g
Maricha - Pepper - (*Piper nigrum*) - Fruit - 96 g
Pippali - Long pepper - (*Piper longum*) - 96 g
Twak - Cinnamon - (*Cinnamomum zeylanicum)* - 192 g
Ela - Cardamom - (*Elettaria cardamomum*) - 192 g
Patra (*Cinnamomum tamala*) - leaves – 192 g
Priyangu (*Callicarpa macrophylla*) - Flower - 192 g
Nagakeshara (*Mesua ferrea*) - Stamen - 96 g
Aswagandharishtam was procured from Standard Ayurvedic Vendors at Chennai.

The drug was subjected to antioxidant assays, namely reducing power, DPPH, and ABTS assays.

**Reducing power assay [31]**
Various concentrations of the Aswagangharishtam in 1 ml of 10% DMSO solution was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding sample or standard. Ascorbic acid at various concentrations was used as reference standard. Increased absorbance of the reaction mixture indicates an increase in reducing power.

% inhibition = [Aswagandharishtam-Control/Aswagandharishtam] × 100

**DPPH radical scavenging assay [32]**
The method described by Oyedemi and Afolayan *et al.* (2011) was used to determine the DPPH scavenging activity of the Aswagandharishtam. The solution of 0.135 mM DPPH was prepared in methanol. Different concentrations of the medicine (0.1 ml) were mixed with 1.9 ml of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference drug. The ability of the medicine to scavenge DPPH radical was calculated from the following formula:

% DPPH inhibition= [(OD of Control - OD of Aswagandharishtam)/(OD of Control)] × 100

**ABTS radical scavenging assay [33]**
A stock solution of ABTS radical cation was prepared by dissolving ABTS (7 mM, 25 ml in deionized water) with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µL). The mixture was left to stand in the dark at room temperature for 15–16 h (the time required for the formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the previous solution and diluting it in ethanol to obtain the absorbency of 0.700±0.02 at 734 nm. The solvent extracts and Aswagandharishtam (0.1 ml) at different concentrations were mixed with the ABTS working solution (1.9 ml), and the reaction mixture was allowed to stand at room temperature for 20 min; then, the absorbance was measured using a ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity is given as ABTS radical scavenging effect that is calculated by the following equation:

ABTS radical scavenging effect (%) = [(OD of Control - OD of Aswagandharishtam)/(OD of Control) × 100

**RESULTS**

Table 1 shows the reducing power assay results. Table 2 shows the DDPH assay results and Table 3 shows the ABTS assay results. Ascorbic acid was used as a standard to compare the antioxidant activities of different assays as shown in Table 4. The comparative percentage inhibition of the three assays as compared to ascorbic acid is summarized in Fig. 1. The comparative $IC_{50}$ values (Fig. 2) indicate that Aswagandharishtam exhibits excellent antioxidant capacity for all the three assays conducted and this could be a very important factor for the medicinal role of Aswagandharishtam.

**Reducing power assay**
The reducing power assay of Aswagandharishtam indicated good antioxidant properties as seen by its $IC_{50}$ value as compared with that of ascorbic acid being shown in Fig. 1.

**DDPH assay**
The DPPH assay of Aswagandharishtam also indicated good antioxidant properties as seen by its $IC_{50}$ value as compared with that of ascorbic acid being shown in Fig. 1.

**ABTS assay**
The ABTS assay of Aswagandharishtam also indicated good antioxidant properties as seen by its $IC_{50}$ value as compared with that of ascorbic acid being shown in Fig. 1.

**Ascorbic acid**
Ascorbic acid was used as a standard to compare the antioxidant activities of different assays as shown in Table 4.

**DISCUSSION**

The present work was in continuation of our studies on the gas chromatography-mass spectrometry (GC-MS) and antioxidant profiles of various Ayurvedic medicines. Two more Aristaas, studied by us, namely Ashokarishtam and Partharishtam, also indicated strong antioxidant properties as was understood by the biomolecules present as shown in the GC-MS analysis [34-36].

The GC-MS analysis study of a similar medicine, Ajaswagandhadi lehyam, in which the major component is Aswagandha (Winter cherry/Indian Ginseng (root) - *W. somnifera*). Withania is reported to medicinal values such as immunomodulator, aphrodisiac, antitumor, anti-inflammatory, anti-stress, antioxidant, sleep-inducing, effective in memory-related conditions, insomnia, hemopoietic effect on CNS, and cardiopulmonary systems [37]. The phytoconstituents present in this plant such as Withanoside IV or VI produced dendritic outgrowth in normal cortical neurons of isolated rat cells, whereas axonal outgrowth was observed in the treatment with withanolide A in normal cortical

**Table 1: The reducing power assay results of Aswagandharishtam**

| S. No | Concentration (µg/ml) | Absorbance | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 1 | 5 | 0.507 | 0.507 | |
| 2 | 10 | 0.528 | 0.528 | |
| 3 | 20 | 0.545 | 0.545 | |
| 4 | 50 | 0.58 | 0.58 | |
| 5 | 100 | 0.623 | 0.623 | |
| | Control | 0.475 | | |
| n | | | 5 | |
| Mean±SD | | | 0.5566±0.045741666 | 250.142 |

SD: Standard deviation

**Table 2: The DPPH assay results of Aswagandharishtam**

| S. No | Concentration (µg/ml) | % Absorbance | Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 1 | 5 | 0.737 | 17.65 | |
| 2 | 10 | 0.681 | 23.91 | |
| 3 | 20 | 0.638 | 28.72 | |
| 4 | 50 | 0.533 | 40.45 | |
| 5 | 100 | 0.482 | 46.14 | |
| | Control | 0.895 | | |
| n | | | 5 | |
| Mean±SD | | | 31.373±11.74214333 | 103.607 |

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, SD: Standard deviation

**Table 3: The ABTS assay results of Aswagandharishtam**

| S. No | Concentration (µg/ml) | % Absorbance | Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 1 | 5 | 0.579 | 12.27 | |
| 2 | 10 | 0.498 | 24.55 | |
| 3 | 20 | 0.485 | 26.52 | |
| 4 | 50 | 0.461 | 30.15 | |
| 5 | 100 | 0.443 | 32.88 | |
| | Control | 0.666 | | |
| n | | | 5 | |
| Mean±SD | | | 25.274±7.949957862 | 197.79 |

ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid. SD: Standard deviation

**Table 4: The ascorbic acid antioxidant profile**

| S. No | Concentration (µg/ml) | % Absorbance | Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 1 | 5 | 0.676 | 24.47 | |
| 2 | 10 | 0.474 | 47.04 | |
| 3 | 20 | 0.33 | 63.13 | |
| 4 | 50 | 0.212 | 76.31 | |
| 5 | 100 | 0.179 | 80 | |
| | Control | 0.895 | | |
| n | | | 5 | |
| Mean±SD | | | 58.19±22.85702846 | 19.59 |

SD: Standard deviation

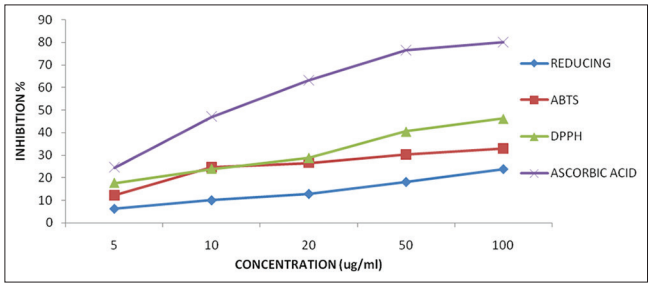


**Fig. 1: The comparative inhibition percentages of all the three assays as compared to ascorbic acid (standard)**

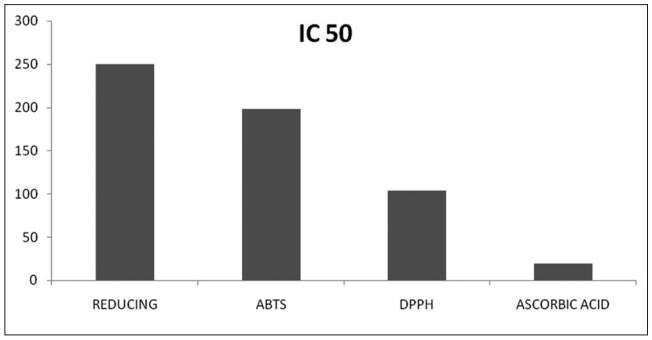


**Fig. 2: The $IC_{50}$ value comparison of all the three assays as compared to ascorbic acid (standard)**

neurons [38]. The crude extract of the plant containing the steroidal substances sitoindosides VII-X and withaferin A augmented learning acquisition and memory in both young and old rats [39].

The present medicine in study, i.e. Aswagandharistam also contains Aswagandha as a major component. Among the constituents of Aswagandharishtam, some have been reported to have strong antioxidant potentials such as C. tuberosum (Baker), *R. cordifolia, T. chebula, B. aistata, G. glabra, P. tuberose, Operculina turpethum, H. indicus, P. santalinus, Z. officinalis, P. longum, P. nigrum, C. tamala,* and *M. ferrea* L [40-56]. Thus, the antioxidant properties as shown in this present work augur well with similar activities of the majority of its constituents.

## CONCLUSION

From the above discussion, it is clear that aristaas, in general, have antioxidant properties and Aswgandharishtam shows very good antioxidant activities with respect to all the three assays, namely reducing assay, DPPH assay, and ABTS assay, proving its efficacy as a potent medicine.

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## AUTHORS' CONTRIBUTIONS

The planning and guidance for this work was done by M.R.K. Rao and K. Prabhu. The experiment was conducted by M. Kotteswari and Siva

Kumar. Sampad Shil was involved in preparing the graphs and analysis of results. All the authors have approved the article.

**CONFLICTS OF INTEREST**

The authors declare that no conflict of interest exists among them.

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