

INVESTIGATION OF EXPECTORANT AND ANTIBACTERIAL POTENTIAL OF MASHI (AN AYURVEDIC DOSAGE FORM) PREPARED FROM ROOTS OF WITHANIA SOMNIFERA L.

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ABSTRACT

Withania somnifera L. (WS) (Ashwagandha), Solanaceae is a nontoxic herb considered as Rasayana in Ayurveda. The ash which is obtained by heating natural products below 450°C at lower temperature and before production of white ash (bhasma) generally called as Mashi. The study was aim to investigate expectorant potential of aqueous and ethanolic extracts of WS roots and Mashi. The expectorant potential of WS Mashi was supported on primary ground by antibacterial activity. WS Mashi was prepared by Ayurvedic procedures viz. Anter-Dhum (internal heat) and Bahir-Dhum (external heat) Paddhati. The previously standardized powdered roots of WS and prepared WS Mashi both were extracted by water decoction and successive soxhlet extraction process for 12-15 hrs using ethanol. Expectorant potential of extracts were evaluated by the phenol red excretion quantities in mice. Optical density values of phenol red solution were measured with 430 nm. Antibacterial activity of WS Mashi was carried out by agar well cup diffusion method against *B. subtilis* and *S. aureus* and *E. coli*. Acute toxicity study showed that all animals are safe without any toxic symptoms up to the dose 2000 mg/kg. The aqueous extract of WS Mashi prepared by Anter-Dhum Paddhati was found to possess most significant expectorant activity (1.8476 ± 0.0450). The ethanolic extract of WS Mashi demonstrate zone of inhibition in plate containing *B. subtilis* and *S. aureus*. The Mashi prepared from WS roots can be used effectively in treatment of cough and bacterial infections.

KEYWORDS: Ashwagandha, Mashi, Anter-Dhum Paddhati, Bahir-Dhum Paddhati, expectorant activity.

INTRODUCTION

Public interest in complementary and alternative medicine is increasing because of its effectiveness in treatment of chronic diseases and due to the increasing side effects and cost of synthetically produced drugs [1]. About 70-80% of the world populations are depends upon traditional/herbal medicines in their healthcare treatment. As well World Health Organization (WHO) encourages, recommends and promotes the use of traditional/herbal remedies in national health care programs because of its easy availability, economical and less or no side effects [2]. Ayurvedic system of medicine is generally considered as one of the oldest Indian system of medicine which is based upon use of plants or animals extract and minerals both in single and compound formulations [3]. Almost 5000 years ago, the description of various dosage forms (solid, liquid or semisolid) have been mentioned in Ayurveda which can be make compatible without losing the potency/efficiency of the drugs [4].

Mashi (black ash) is a solid Ayurvedic dosage form in which bulk of raw material (any natural product from vegetable or animal sources) is reduced to a greater extent by application of a certain quantum of energy below 450°C [5]. The process is called as 'masikalpana' in Ayurveda. Mashi retains both organic and inorganic constituents (hidden therapeutically active chemical constituents become prominent) and provides 'sendriyatva' (potency). The black colour and charcoal like taste of

Mashi attributed to the high percentage of oxides, inorganic elements and carbon [6]. The organic biomolecules like proteins and long-chain carbohydrates begin to break down into their respective monomeric units on combustion as well as [free radicals](#) generated from lipids speed up the breakdown of other biomolecules [7]. The reduction depends on the type of method used to prepare the Mashi and also on how accurately the procedure is followed. According to the Ayurvedic literature Mashi can be prepared by two different methods viz. Bahir-Dhum Paddhati (also called as external heat method in which material is heated in an open earthen pot) or Anter-Dhum Paddhati (also called as internal heat method in which material is heated in closed earthen pot) [6,7]. The various Mashi formulations prepared from plant as well as from animal source are studied for their therapeutic applications which can be used for both internal and external purpose viz. Ashwagandha Mashi, Triphala Mashi, Haritaki Mashi, Hastidant Mashi, Mayurpiccha Mashi, Sarpa Mashi etc. [7]. Withania somnifera L. (WS) (Ashwagandha) from the family Solanaceae is a nontoxic herb of traditional system of medicine considered as Rasayana (nutritious at all levels) in Ayurveda. It is commonly known as 'Indian Winter cherry' which is used for the treatment of various kinds of diseases especially as a nerve tonic. It enhances the function of the brain and nervous system and improves the memory [8]. In present research work WS Mashi was prepared as per the procedure mentioned in our previous study [6] and it was evaluated for its expectorant potential by phenol red excretion method and qualitatively for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Procurement, authentication and processing of WS roots

The dried mature roots of WS were procured from local market of Pune (Maharashtra), India. The procured plant material was authenticated from Agharkar Research Institute, Pune (herbarium voucher no.- Auth.12-102). The collected fresh plant material was thoroughly cleaned and air-dried, homogenized to fine powder and stored in air-tight bottles separately until used [9, 10].

Preparation of WS Mashi

WS Mashi was prepared by using Ayurvedic procedures viz. Anter-dhum Paddhati (internal heat), Bahir-dhum Paddhati (external heat) (**Supplementary procedure 1**) [6, 7].

Preparation of extracts of WS roots and Mashi

The previously standardized powdered roots of WS [6, 7] and prepared WS Mashi were extracted by water decoction process and by continuous successive soxhlet extraction process for 12-15 hrs with ethanol [11]. After complete removal of solvents, the dried extracts thus obtained were stored in appropriate container for further evaluation.

EVALUATION OF EXPECTORANT POTENTIAL OF EXTRACTS OF WS ROOTS AND MASHI

Procurement and maintenance of animals

Swiss albino mice were obtained from National Institute of Biosciences, Pune (Maharashtra) and housed in polypropylene cages and maintained at 24±2°C under 12h light/dark cycle and were fed ad libitum with standard pellet diet and had free access to water at animal house of S.G.R.S. College of Pharmacy, Saswad, Pune. The experimental protocol was approved from the Institutional Animal Ethics Committee (SGRS/IAEC/11/2011-12) and animals handling was performed according to CPCSEA guidelines [12].

Acute toxicity study

Swiss albino mice of either sex weighing between 20±5 g were selected for acute toxicity study as per the OECD guidelines 425 [13]. LD₅₀ value was obtained for both extracts by counting the death and alive mice while performing each test for maximum 48 hrs after treatment. Five mice were randomly selected and marked for individual identification. Animals were fasted 24 hrs prior to dosing (administered in a single dose orally). Five animals were treated with a starting dose of 2000 mg/kg and one animal is treated with 5000 mg/kg B.W. Animals were observed individually after dosing at least once during the first 30 minutes periodically during the first 24 hrs, with special attention given during the first 4 hrs. [14].

Expectorant activity

Swiss albino mice of either sex weighing between 20±5 g were divided into fifteen groups (n=6). The control group received saline; positive control group received ammonium chloride; standard group treated with marketed formulation TUS-Q and other test groups were treated with aqueous and ethanolic extracts of WS roots and Mashi (Anter-dhum and Bahir-dhum). (Supplementary Table 1)

The animals were housed under the standard laboratory conditions (light period of 12 h/day, temperature at 25±2°C and humidity 55±5%) with free access to food (standard pellets chow, Lipton, India) and water ad libitum. Food but not water was deprived overnight during the experiment. The experiment was carried out according to the CPCSEA guidelines [15]. The test substance is administered in a single dose by gavages using an intubation cannula.

Phenol red assay method

The expectorant activity of aqueous and ethanolic extracts of WS roots and Mashi was carried out by phenol red assay method. The animal groups (n=6) were received single dose of test drugs by oral administration for three days respectively.

One hour after the last drug administration, 5% of phenol red in saline (500 mg/kg) were injected into the abdomen of mice and then the mice were killed after 30 min. The windpipe of the dead mice were peel off and then put into 3.0 ml of saline. 0.3 ml NaOH (1 mol/L) was added and mixed for 30 min. The optical density (OD) values of solutions were measured on a spectrophotometer with the wavelength of 430 nm. The excretion of phenol red was determined according to the standard curve [16].

Statistical evaluation

The data of expectorant animal activity of aqueous and ethanolic extracts of WS roots and Mashi was statistically evaluated by one way ANOVA followed by Dunnet's multiple comparison tests by considering $p < 0.05$ statistically significant value.

EVALUATION OF ANTIBACTERIAL POTENTIAL OF EXTRACTS OF WS ROOTS AND MASHI

Procurement of micro-organisms

The gram-positive bacteria viz. *Bacillus subtilis* and *Staphylococcus aureus* and gram-negative bacteria viz. *Escherichia coli* were employed for in-vitro antibacterial activity. The stock cultures of test micro-organisms were procured from National Chemical Laboratory (NCL), Pune (Maharashtra) and maintained at microbiology departments, PDEA's Waghire College of Science, Saswad, Pune.

In-vitro antibacterial activity

The in-vitro study of antibacterial activity of ethanolic, methanolic and aqueous extracts of WS Mashi prepared by Anter-dhum Paddhati was carried out by agar well cup diffusion method [17].

The plates were sterilized by dry heat in an oven at 160°C for one hour. The agar medium was prepared, sterilized in an autoclave at 121°C, 15 lbs pressure for 15 minutes and then poured in petri plate aseptically and allowed to solidify. In the meantime tubes containing standard culture of microorganisms was inoculated and mixed well. After solidifying the plates, wells of 6 mm were bored aseptically using sterile cork borer. The holes were filled completely with desired extract. The plates were kept in refrigerator for proper diffusion of added extracts. The plates were incubated at 37°C for 24 hrs and then plates were observed for zone of inhibition. Amoxicillin was used as a positive control [18].

RESULTS AND DISCUSSION

Withania somnifera L. (WS) (Ashwagandha) is also known as 'Sattvic Kapha Rasayana' (expectorant) herb [8]. The use of WS ash along with ghee and honey has been reported in Ayurveda for the treatment of cough. Our previous study shows that WS roots and Mashi contains alkaloids, steroids, saponin glycosides and carbohydrates in aqueous and ethanolic extracts, but the intensity of presence of active constituents was found to be high in ethanolic extracts [6]. These active phytoconstituents of WS could be responsible for therapeutic properties of Ashwagandha.

The acute toxicity study exhibited that all animals were safe without any toxic symptoms up to the dose 2000 mg/kg of WS roots and Mashi extracts. This dose was taken maximum tolerable dose and 1/10th dose of maximum tolerable dose was calculated (200 mg/kg). The 100 mg/kg and 200 mg/kg were selected and doses were calculated as per the body weights of animals for further study.

Expectorant effect of aqueous and ethanolic extracts of WS roots and Mashi was evaluated by the phenol red excretion quantities in mice (Table 1). Optical density values of phenol red standard solution were measured with the wavelength of 430 nm. The aqueous and ethanolic extracts of WS Mashi were found to possess significant expectorant activity when compared with animal groups treated with ammonium chloride (positive control) and TAS Q (marketed formulation) (Supplementary Figure 1 and 2)

Table 1. Expectorant activity of aqueous and ethanolic extracts of WS roots and Mashi (prepared by Anter-dhum and Bahir-dhum Paddhati) by phenol red assay method.

Group →	Control group (Saline)	Positive control group (Ammonium Chloride; 1 mg/kg)	Standard group (Marketed formulation TUS-Q; 5 mg/kg)	Test groups											
				Aqueous extract					Ethanolic extract						
				WS roots		WS Mashi Prepared by Anter- dhum Paddhati		WS Mashi Prepared by Bahir- dhum Paddhati		WS roots		WS Mashi Prepared by Anter- dhum Paddhati		WS Mashi Prepared by Bahir- dhum Paddhati	
Parameters ↓				100	200	100	200	100	200	100	200	100	200	100	200
Optical	0.05 79 ±	0.0905 ±	0.0732 ±	0.0 627	0.0 996	0.1 094	0.1 275	0.0 703	0.0 952	0.0 674	0.0 866	0.1 120	0.1 234	0.1 033	0.1 061

density	0.00 09	0.0008	0.0018	±	±	±	±	±	±	±	±	±	±	±	±
				0.0 019	0.0 024	0.0 021	0.0 031	0.0 019	0.0 019	0.0 017	0.0 017	0.0 013	0.0 012	0.0 010	0.0 021
Phenol red excretion on mg/l	0.83 97 ± 0.01 30	1.3126 ± 0.0123	1.0616 ± 0.0258	0.9 095	1.4 447	1.5 858	1.8 476	1.0 189	1.3 792	0.9 772	1.2 559	1.6 236	1.7 878	1.4 967	1.5 376
				±	±	±	±	±	±	±	±	±	±	±	
				0.0 275	0.0 356	0.0 315	0.0 450	0.0 288	0.0 278	0.0 253	0.0 250	0.0 183	0.0 177	0.0 149	0.0 305

Where, WS- *Withania somnifera* Linn.

The phenol red excretion was found to be more in a group treated with aqueous extract of WS Mashī (Anter-dhum Paddhati; 200 mg/kg) i.e. 1.8476 ± 0.0450 mg/l. Both, aqueous and ethanolic extracts of WS Mashī prepared by Anter-dhum Paddhati had significant expectorant activity. This could be due to the presence of high percentage of oxides, inorganic elements and carbon in the WS Mashī as well as Anter-Dhum Paddhati (internal heat method) could avoid loss of phytoconstituents from the mixture [6, 7].

The antibacterial activity of WS Mashī was also evaluated qualitatively on primary ground to support the expectorant potential. The results of antibacterial activity (Figure 1) demonstrate that ethanolic extract of WS Mashī shows substantial zone of inhibition in plate containing *B. subtilis* and *S. aureus* but methanolic and aqueous extracts of WS Mashī were found to be devoid of antibacterial activity.

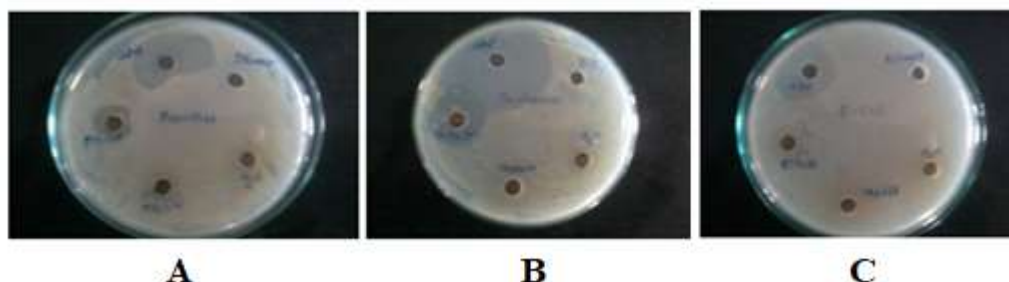


Figure 1. Antibacterial potential of ethanolic, methanolic and aqueous extracts of WS Mashī prepared by Anter-dhum Paddhati. **A-** *B. subtilis*, **B-** *S. aureus*, **C-** *E. coli*.

CONCLUSION

The results of preliminary evaluation of ethanolic and aqueous extracts of WS roots and Mashī for its expectorant and antibacterial potential advocate that, the Mashī (Solid Ayurvedic dosage form) can be used significantly for the treatment of cough and bacterial infections. The expectorant and antibacterial potential of WS roots and Mashī could be due to the presence of phytoconstituents like alkaloids, steroids, glycosides etc. However, the exploration of mechanism of action of phytoconstituents and antibacterial activity against more infectious and specific bacteria is needed.

Declaration of interest

The authors declare that the research was conducted in the absence of conflict of interest. The authors alone are responsible for the content of the paper.

REFERENCES

- Pandey MM, Rastogi S, Rawat AKS. Indian traditional Ayurvedic system of medicine and nutritional supplementation. *Evid Based Complement Alternat Med.* 2013; Article ID 376327.
- Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014; Article ID 177.
- Kumar S, Dobos GJ, Rampp T. The significance of ayurvedic medicinal plants. *Evid Based Complement Alternat Med.* 2017; 22 (3): 494-501.
- Mahanta P, Nidagundi SP, Sobagin MV. A Literature Review on various Ayurveda Dosage forms. *J Ayurveda Integr Med.* 2019; 4 (5): 191-195.
- Biradar YS, Jagatap S, Khandelwal KR, Singhania SS. Exploring of antimicrobial activity of Triphala Mashī - An ayurvedic formulation. *Evid Based Complement Alternat Med.* 2008; 5 (1): 107-113.
- Barke SA, Woyal SR, Abhyankar MM, Patil RY. Preparation and pharmacognostic evaluation of Ashwagandha Mashī. *J Pharmacogn Phytochem.* 2018; 7 (3): 2499-2505.
- Joshi A, Baheti A, Wani M, Nimbalkar R. A review through therapeutic attributes of Ayurvedic formulation mashī. *J Ayurveda Integr Med.* 2021; 12 (4): 715-721.
- Ayurveda College. The Characteristics, Benefits and Application of Ashwagandha in the West. [Accessed 2019]. Available from: <https://www.ayurvedacollege.com/wp-content/uploads/2017/06/The-Characteristics-Benefits-and-Application-of-Ashwagandha-in-the-West-By-Tanya-Gardner.pdf>
- Woyal SR, Gurav SS. Pharmacognostic and phytochemical investigation of potentially important plants of Western Ghats, India. *Int J Pharm Sci Res.* 2019; 10 (6): 3101-3108.
- Woyal SR, Gurav SS. Bhallatakadi ghrita: development and evaluation with reference to murcchana and shata-dhauta

- process. *J Ayurveda Integr Med.* 2020; 11:261-269.
11. Mukherjee PK. *Quality Control of Herbal Drugs an Approach to Evaluation of Botanicals*. 3rd ed. Delhi: Business Horizons Pharmaceutical Publishers, 2008.
 12. Wayal SR, Gurav SS. Evaluation of wound healing potential of Bhallatakadi Ghrita–cow ghee based polyherbal formulation: in-vivo excision and incision wound model. *J Complement Integr Med.* 2021; 18 (3): 507-515.
 13. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. [Accessed 2020]. Available from: <https://www.oecd.org/env/test-no-425-acute-oral-toxicity-up-and-down-procedure-9789264071049-en.htm>
 14. Ghosh MN. *Fundamentals of Experimental Pharmacology*. 4th ed. Hilton & Company, 2008.
 15. Wayal SR, Barke SA, Nigade G, Jagtap P, Gurav NS, Prasad S, Wanjari M, Ayyanar M, Gurav SS. Effect of “Murcchana samskara” on therapeutic efficacy of Bhallatakadi Ghrita. *J Ayurveda Integr Med.* 2022; Article ID 100547.
 16. Vogel HG, Scholkens B, Bernward A, Sandow J, Muller G. *Drug Discovery and Evaluation, Pharmacological Assays*. 2nd ed. New York: Springer, 2002.
 17. Gurav SS, Deshkar NS, Tilloo SK, Duragkar NJ, Burade KB. Antimicrobial and antioxidant evaluation of *Flacourtia ramontchi* L. Herit. *J Herbs Spices Med Plants.* 2013; 19: 76-95.
 18. Mehrotra V, Mehrotra S, Kirar V, Misra K, Srivastava AK, Nandi SP. Antioxidant and antimicrobial activities of aqueous extract of *Withania somnifera* against methicillin-resistant *Staphylococcus aureus*. *J Microbiol Biotechnol Res.* 2011; 1 (1): 40-45.

ABBREVIATIONS

B.W. - Body Weight

CPCSEA - Committee for the Purpose of Control and Supervision of Experiments on Animals

LD₅₀ - Half Lethal Dose

NCL - National Chemical Laboratory

OD - Optical Density

OECD - Organization for Economic Co-operation and Development

WS - *Withania somnifera* L.