



PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY OF METHANOL AND WATER EXTRACT OF *WITHANIA SOMNIFERA* (ASHWAGANDHA CHURNA)

S. R. Sonwane^{1*}, M. O. Malpani¹, A. R. Lokhande²

¹ Department of Chemistry, Shankarlal Khandelwal College, Akola 444 002 (M.S), India.

² Department of Microbiology, Shankarlal Khandelwal College, Akola 444 002 (M.S), India.

*Email: shraddhasonwane49@gmail.com

ABSTRACT

The given study focused on the phytochemical determination and antibacterial activity of Ashwagandha (churn), an important traditional medicinal plant in India. The antibacterial activity of Methanol and Water extract was studied by the Disc diffusion method (Kirby-Baur Method) against pathogenic bacterial strains of *Staphylococcus aureus*. Among the solvent used water is used for the extraction of tannin and methanol is used for the extraction of flavonoids. Phytochemical analysis was done by chemical test. The methanol extract does not inhibit both strains of *Staphylococcus aureus* while water extract inhibits the growth of both strains of *Staphylococcus aureus*. The results provided evidence that the churn might have a potential source of phytochemical constituents that act as antibacterial agents and can be used as complementary to traditional methods.

Keywords: Phytochemical analysis, Phytochemical extraction, *Staphylococcus aureus*, Antibacterial activity.

INTRODUCTION

Plants are not only a primary source of food and fuel but are also used for folk medication due to the presence of active chemical compounds or secondary metabolites. Due to inadequate health centres, shortage of medicines and low-income communities, almost 70% of people in developing countries are mainly dependent on less-cost plants. For the last few decades, phytomolecules such as alkaloids, tannins and flavonoids extracted from plants are eco-friendly with negligible toxicity and are non-immunogenic. Among these plants, *Withania somnifera*, in the family of *Solanaceae*, is a known medicinal plant grown throughout the world¹. *Withania somnifera*, also known as Ashwagandha, [Indian Ginseng, or Winter cherry, is a plant of the *Solanaceae* or nightshade family. It is used as an herb in Ayurvedic medicine. It grows in dry parts in sub-tropical regions. Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra and Madhya Pradesh are the major Ashwagandha-producing states of the Country². Ashwagandha, the Indian Ginseng or winter cherry has been used as a quite valuable herb in the Ayurvedic and indigenous medical system for over 4000 years. Ashwagandha is considered to be one of the best rejuvenating agents in Ayurveda. Its roots, seeds and leaves are used in Ayurvedic and Unani medicines. Ashwagandha and its extracts are used to prepare herbal tea, powders, tablets and syrups. It has anti-inflammatory, anti-tumour, anti-stress, antioxidant, mind-boosting, immune-enhancing, and rejuvenating properties.

MATERIAL AND METHOD

1. Collection of Ayurvedic drugs or churn

Ashwagandha (*Withania somnifera*) churn is directly purchased from Ayurvedic medicinal stores in the Akola region having the application of infectious diseases.

2. Extraction and Isolation

The 10 grams of Ashwagandha churna was extracted with solvent water by boiling on a Magnetic stirrer for 30 minutes, to extract Tannins. The 10 grams of churna with solvent Methanol using Soxhlet apparatus for Flavonoid extraction. Both the extracts were screened for phytochemical analysis of Tannin and Flavonoid.

3. Phytochemical screening

Test for Tannin (Ferric chloride test) - 0.5gram extract was stirred with 10 ml of distilled water and then filtered. A few drops of 1% ferric chloride solution were added to 2 ml of the filtered and observed for the blue-black, green or blue-green precipitate.

Test for Flavonoid (Lead acetate test) - 50 ml of extract was taken in a test tube and a few drops of lead acetate solution were added to it and observed for yellow-coloured precipitate.

4. Antibacterial assay

The methanol and water extract were examined for their antibacterial potency by Disc diffusion (Kirby-Baur method) against two strains of *Staphylococcus aureus*. The strains were collected from the Department of Microbiology, Shankarlal Khandelwal College, Akola. Petri plates were prepared with 25 ml of sterile Mueller-Hinton agar. And disc was prepared by using Whatman filter paper impregnated with methanol and water extract of given Ashwagandha churna. 1 ml of bacterial suspension uniformly swabbed over the agar medium. The dried impregnated disc was placed on this agar with one negative controlled disc and incubated for 48 hours at 37⁰ C. The presence of a zone of inhibition

surrounding the disc was considered as an antibacterial activity of methanol and water extract. The zone of inhibition was recorded in millimetres by using a zone scale.

RESULT AND DISCUSSION

The result showed that churn has phytochemical constituents in both methanol and water extract. From analysis, it confirmed that tannin and flavonoid are present in water and methanol extract respectively.

Table 1: Phytochemical analysis of Ashwagandha

Sr. No	Phytochemical analysis by chemical method	Extracts	
		Ashwagandha Aqueous	Ashwagandha methanol
1.	Lead acetate test for flavonoid	–	Positive
2.	Ferric chloride test for Tannin	Positive	–

Fig 1: Extraction of Tannin and Phytochemical Analysis

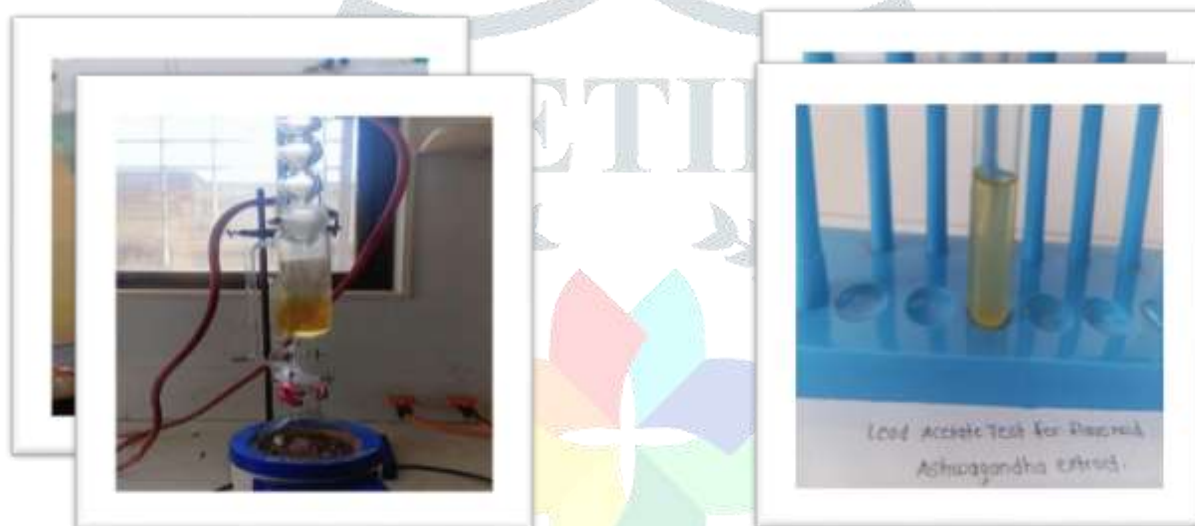


Fig 2: Extraction of Flavonoid and Phytochemical Analysis

Table 2: Antibacterial activity of Phytochemical constituents

Antibacterial activity of these phytochemical constituents on the Mueller-Hinton agar plate shows the zone of inhibition measuring 14 mm and 13 mm for tannin against strains one and two of *Staphylococcus aureus*, while flavonoid does not exist any zone of inhibition it reveals that tannin can inhibit the growth of *Staphylococcus aureus*. It indicates that Ashwagandha churna is used as an antibacterial agent as a comparative standard medicine.

There are many reports in the literature regarding antibacterial activity and Phytochemical analysis. Jinu John et al. 2014 studied a detailed survey on various traditional uses, phytochemical composition and pharmacological properties of *Withania somnifera*. In the study of Bashir et al. 2013, the methanolic crude extract of *Withania somnifera* leaves (Solanaceae) was screened for Phytoconstituents, and the extracts revealed the presence of flavonoids, steroids, alkaloids, saponins and tannins. Munnaza Aqeel et al. 2010 investigated, the efficient antibacterial activity of *Withania somnifera* root extract against *Staphylococcus aureus* and *Escherichia coli*. *Withania somnifera* showed the highest zone of inhibition in culture plates.

ACKNOWLEDGEMENT

Sr. No.	Extract	Phytochemical Present	Antibacterial activity (Zone of inhibition in mm)	
			Strain 1	Strain 2
1.	Ashwagandha in methanol extract	Flavonoid	No zone	No zone
2.	Ashwagandha in water extract	Tannin	14 mm	13 mm

We are thankful to Principal, Shankarlal Khandelwal Arts, Science, and Commerce College Akola, SGBAU University, Maharashtra, India, for making the laboratory facility available for the research work. And also thankful to the Department of Chemistry & Department of Microbiology, Shankarlal Khandelwal College, Akola (M.S), India.

REFERENCES

1. Aqeel M, Alam S S, Abbas K, and Abdul Hameed Khan. A Comparative Antibacterial Study of Withania somnifera Root Extract with Antibiotics against Escherichia coli and Staphylococcus aureus. S.Z.P.G.M.I. Vol: 25(1): pp. 49-57, 2010.
2. Bashir, H. S.; Mohammed, A. M., Magsoud, A. S., Shaoub, A. M. Isolation of Three Flavonoids from Withania Somnifera Leaves (Solanaceae) and their Antimicrobial Activities. JOURNAL OF FOREST PRODUCTS & INDUSTRIES, 2013, 2(5), 39-45 ISSN:2325.
3. Dessie E., Natinael M., and Molla T., Phytochemical analysis of Withania somnifera leaf extracts by GC-MS and evaluating antioxidants and antibacterial activity. INTERNATIONAL JOURNAL OF FOOD PROPERTIES 2023, VOL. 26, NO. 1, 581–590.
4. Dharajiya D, Patel P. Patel M., Moitra N. In vitro Antimicrobial Activity and Qualitative Phytochemical Analysis of Withania somnifera (L.) Dunal Extracts. Int. J. Pharm. Sci. Rev. Res., 27(2); (2014).
5. Jinu John. Therapeutic potential of Withania Somnifera: A report on phytopharmacological properties. International Journal of Pharmaceutical Sciences and Research 2014; Vol. 5(6): 2131-2148.
6. Khidir A. and Abdel Karim, M. Constituents and Antimicrobial Activity of Oil from Withania somnifera Grown in Sudan. Journal of The Faculty of Science and Technology (JFST), Issue No. 9 (2) (2022) 117 – 124.
7. Latifa Nasser A. Abdulqawi. In vitro antibacterial and antioxidant activities of Withania somnifera L. Extract. International Journal of Research and Analytical Reviews 2020 IJRAR, Volume 7, Issue 1.
8. Mali P. and Singh A., Isolation, characterization and evaluation of the antimicrobial activity of Withanolide-A of Withania somnifera. International Journal of Pharmacological Research IJPR Volume 3 Issue 3 (2013).
9. Santhi, M and Swaminathan, C. Evaluation of antibacterial activity and phytochemical analysis of leaves of Withania somnifera (L.) Dunal, International Journal of Current Research Vol.33, Issue, 3, pp.010-012, March, 2011.
10. Singariya P., Kumar P. and Dr. Mourya K.K. Comparative Pharmacological Study of Fruits and Flowers Extract of Withania somnifera. Int. J. Pharm. Phytopharmacol. Res. 2012, 1(5): 276-282.
11. Solanki S, Prasad D, Singh A K, Antioxidant Determination and Thin Layer Chromatography of Extract *Withania Somnifera*. European Journal of Molecular & Clinical Medicine ISSN 2515-8260 Volume 7, Issue 11, 2020.
12. Swaminathan C., M. Santhi. Phytochemical Analysis and Antimicrobial Activity of Roots of Withania somnifera (L.) Dunal. International Journal of Chem Tech Research, 2019,12(2): 218-222.