REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 22 April 2023 Revised: 18 May 2023 Accepted: 23 June 2023

In vitro studies of Withania somnifera plant extract with selected Antibiotics

Niharika Jegoda[1], Vikram Panchal[2], Namrata Patel[3], Kinjal Patel [4] and Piyush J. Vyas[5]

[3,4&5]Department of Chemistry, Sheth M. N. Science College, Patan-384265, Gujarat, India.
[1&2]Department of Chemistry, Gokul Science College, Sidhpur-384151, Gujarat, India.
E-mail: vyaspiyushj@yahoo.com

ABSTRACT
Antibiotics play a major role in clinical medicine in eradicating pathogens. The arrival of genuine strains of bacteria has seriously limited our talent to treat bacteria and selected antibiotics are dreadfully desirable. In this study, the plant extracts were prepared by using diff. solvents (Acetone/Ethanol/Methanol). Antimicrobial evaluation of plant extracts, some particular Antibiotics like Amoxicillin, Ceftazidime, Ciprofloxacin, Amphotericin-B and Fluconazole and combinations has been done by Agar well Diffusion method. The results of this study displayed that ethanol and methanol extract show higher activity against nominated pathogenic species. HPTLC has been also achieved to check if there any new compound formed or not by amalgamation of extract and antibiotics. The findings of this study suggest that coalescing plant extract with antibiotics may be effective in battling newly developed drug-resistant microbes.

KEYWORD: Withania somnifera Extract, HPTLC, Antibiotics, Antimicrobial Activity

INTRODUCTION

Plant extract complexes and their claim in food industries have great value in avoiding progress of fungi or bacteria, and can be elaborate in possible giving out knowledge in which they can be oppressed as ideal preservative explanations. The highlighted assistances and trials of plant-derived products need further enquiry for green society execution and governmental parameter.[1,2] Upward regulatory margins and negative buyer responses to living compounds and to the usage of antibiotics in agriculture have underwritten to heaviness for the progress of alternative compounds for use as antimicrobial mediators.[3-5]In outmoded medicine, Withania somnifera has been real as having antitumor, anti-inflammatory, antimicrobial, antioxidant, antiviral, and hepatoprotective, and many other activity.[6,7] Bioactive complexes such as ashwagandhine, cuscohygrine, sitoindosides, anhygrine, withanamides, and tropine have been secluded and identified from W. somnifera.[8-11] In calculation, the most central withanolides, withaferine A and withanolide A, have been isolated. The plant has publicized the presence of chemically active amalgams related to flavonoids, alkaloids, terpenoids, steroidal lactones, and saponins in the citations from parts.[12-14] Fatty acids such as palmate, oleic, linoleic, and linolenic acids have been isolated from n-hexane extracts of verdures and roots.[15] In some cases, W. somnifera extracts with multi-components have shown better medicinal extract than the sanitized complexes.[16]

Through recent years, research copies have familiarized soft rot bacteria as a dangerous pathogen that could extinguish many horticulture collects, and have established methods to recognize or characterize it, and unfluctuating to afford control tactics.[17,18] Dickey spp. strains have been isolated from diseased plants in Finland, Poland, France, the Netherlands, Switzerland, and other European fatherlands, while Egypt and Israel have been connected with Serratia pylumthica.[19-21] D.

solani strains are painstaking more aggressive than other blackleg-causing microbes.[22,23] Erwinia amylovora, a Gram-negative bacterium, is the causal agent of fire blight.[24]Hence, in this work we weigh the antimicrobial activity of particular antibiotics frozen with W. somnifera plant extract in contrast to some plant pathogenic bacteria and fungi. This study also analyzes the chemical conformations of new compound fashioned or not by using HPLC analysis.

MATERIAL AND METHOD

Collection of Plant Material

Garden-fresh W. somnifera were acquired from different area of Northern Gujarat. The separate models were sorted to remove bad quality, soaked in tap water, washed and rinsed under seriatim water. They were cut, dried, powdered, stored in antiseptic condition and used for further scholarships.

Extraction

In a round-bottom flask with a magnetic stirrer, 30 grams of residue of was extracted for 6 hours, 12 hours, and 24 hours with 120 ml of the methanol, ethanol, and acetone solvent to square the result of extraction time on the removed profit.

Time	Methanol	Ethanol	Acetone
6 hours	1.125 gm	0.997 gm	0.850 gm
12 hours	1.322 gm	1.227 gm	0.967 gm
24 hours	1.775 gm	1.430 gm	1.218 gm

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 22 April 2023 Revised: 18 May 2023 Accepted: 23 June 2023

Preparation of combination
The combination was prepared by coalescing Antibiotics with diff. extracts. Antibiotic and plant extract were kept at a 1:1 ratio.

Antimicrobial evaluation
Agar well diffusion method optional by Arodiya et al. used to evaluate the antimicrobial activity of W. somnifera extracts, Antibiotics, and their Combinations. Agar media was prepared by using Muller Hinton Agar. The agar plate surface was inoculated by spreading the selected microbial (S. aureus, B. subtilis, P. aeruginosa, E. coli, A. niger and C. albicans) over the entire agar surface. Then, wells, a diameter of 8 mm were punched with a sterile cork borer and 50 µL of the tested solution at desired concentrations introduced into the well. Then, agar plates were incubated at 37 °C for 24 hrs. The zone of inhibitin was used to express the antimicrobial activity in mm.

HPTLC
Prewashing of HPTLC plates
HPTLC plates (10 cm×10 cm) were washed with methanol and activated at 120 °C for 15 min using a TLC plate heater III.

Preparation of Standards
Standard solutions of W. somnifera methanol extract, antibiotic and their combinations were set with methanol at a concentration of 0.1 mg/mL. A mixture of the ethics was also arranged by combining equal volumes of each standard solution.

Plate development and derivatization
The Linomat 5 semi-autosampler was used to apply the separate standard solutions and standard mixture as 2-lL bands in five tracks, 1 cm from the plate's base, with a bandwidth of 5 mm and layout between bands of 2 mm. All tracks 1-5 on the plate's customary samples in the resulting order: antibiotic, methanol extract, antibiotic + methanol extract, ethanol extract, and antibiotic + ethanol extract. 10 mL of mobile phase were pre-saturated in HPTLC twin trough chambers (10 cm x 10 cm) for 15 minutes. Over a migration distance of 5 cm, the mobile phase was employed to resolve the adsorbed standard and standard mix after being dispersed equally across the twin trough chamber. The mobile phase was composed of ethyl acetate:methanol:acetone:toluene:ammonia (1:5:7:0.5:0.5). At theend, plates well allowed to dry and analysed.

RESULT AND DISCUSSION
W. somnifera extracts were compounded with API and exposed to antimicrobial property. Zone of inhibition is expressed in mm.

Antimicrobial Evaluation
Methanol, ethanol, and acetone extracts of powdered W. somnifera bark were tested for their antibacterial effects on their own and in combination with selected antibiotics (Amoxicillin, Ceftazidime, Ciprofloxacin and Erythromycin). Against tested bacterial species, all three extracts exhibit potent antibacterial activity.
Acetone extract shows zone of inhibition between 5 to 10 mm for respective bacterial culture for 1% w/v (25 µg/ml) concentration ethanol extract shows zone of inhibition between 3 to 8 mm for respective bacterial culture. Methanol extract display zone of inhibition between 8 to 14 mm for particular bacterial strain. **(Table 1)** all three extracts showed intense antibacterial activity against E. coli species.

Amoxicillin significantly reduced the growth of S. aureus and B. subtilis, its combination with all three extracts also exhibits significant growth inhibition against these two species among all examined bacteria. A combination of methanol extract with amoxicillin shows 35 mm, 34 mm, 29 mm, and 39 mm of ZOI against S. aureus, B. subtilis, P. aeruginosa, and E. coli, respectively, at 1% w/v concentration. The combination of amoxicillin with methanol and ethanol extracts also showed higher activity than with acetone extract, similar to pure extracts **(Table 2)**.

Ciprofloxacin when combine with extract it is showed that the combination have higher inhibition effect against all stain. All three solvent extract and its combination shows synergic effect against respective bacterial culture **(Table 3)**.
When extract combined with Ceftidizime it has good resistance potential against all four bacterial culture. Pure extract and combination has good resistance potential. As per **Table 4** methanol, ethanol and acetone exctract and its combination have good synergic effect against bacterial culture. 1% w/v solution have good resistance potential against E.coli almost 24 mm to 22 mm.

Erythromycin and its combinations with all three extracts show higher activity against S. aureus and B. subtilis and lower activity against P. aeruginosa. Combination of erythromycin with acetone extract exhibited 27 mm, 24 mm, 9 mm and 21

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 22 April 2023 Revised: 18 May 2023 Accepted: 23 June 2023

mm while ethanol extract exhibited 26 mm, 26 mm, 21 mm, and 23 mm, and with methanol extract show 27 mm, 24 mm, 21 mm, and 21 mm of ZOI against S. aureus, B. subtilis, P. aeruginosa, and E. coli respectively at 1000 µg/ml concentration **(Table 5)**.

As the concentration of all three extracts decreased, the activity of their combinations with used antibiotics also slightly dropped.

Table 1: Antibacterial activity of pure extract

Bacteria	Acetone Extract				Ethanol Extract				Methanol Extract			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	05	05	04	03	08	06	05	04	09	08	04	04
B. subtilis	04	04	04	03	07	05	04	04	08	07	04	04
P. aeruginosa	08	08	07	06	11	09	08	07	12	11	07	07
E. coli	10	10	09	08	13	11	10	09	14	13	09	09

Table 2: Antibacterial activity of extract with Amoxicillin

Bacteria	Acetone Extract + Amoxicillin				Ethanol Extract + Amoxicillin				Methanol Extract + Amoxicillin			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	34	34	33	31	34	33	31	30	35	35	30	29
B. subtilis	31	30	30	28	34	33	31	31	34	33	32	30
P. aeruginosa	16	15	15	13	29	29	26	26	29	28	27	26
E. coli	28	27	27	25	30	30	30	27	29	29	27	26

Table 3: Antibacterial activity of extract with Ciprofloxacin

Bacteria	Acetone Extract + Ciprofloxacin				Ethanol Extract + Ciprofloxacin				Methanol Extract + Ciprofloxacin			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	30	30	29	28	31	31	30	29	30	30	29	28
B. subtilis	32	31	30	30	31	31	30	29	29	28	27	27
P. aeruginosa	27	27	26	25	26	26	25	25	26	25	24	24
E. coli	24	24	23	22	28	28	27	26	26	25	24	24

Table 4: Antibacterial activity of extract with Ceftazidime

Bacteria	Acetone Extract + Ceftazidime				Ethanol Extract + Ceftazidime				Methanol Extract + Ceftazidime			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	27	27	26	25	26	26	25	24	26	24	24	23
B. subtilis	30	30	29	29	30	29	28	28	27	27	26	25
P. aeruginosa	28	28	27	26	30	30	29	29	32	31	31	30
E. coli	24	23	20	20	24	24	23	23	22	21	20	20

Table 5: Antibacterial activity of extract with Erythromycin

Bacteria	Acetone Extract + Erythromycin				Ethanol Extract + Erythromycin				Methanol Extract + Erythromycin			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	27	27	26	24	26	25	24	23	27	25	24	23
B. subtilis	24	24	23	21	26	25	24	23	24	24	23	22
P. aeruginosa	09	09	08	06	21	20	19	18	21	20	20	19
E. coli	21	21	20	18	23	22	21	20	21	21	20	19

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 22 April 2023 Revised: 18 May 2023 Accepted: 23 June 2023

Antifungal Evaluation

All three extracts show effective antifungal activity against the fungus A. niger and C. albicans. Among all three extracts, methanol and ethanol extract showed higher activity against both the tested fungus than acetone extracts. All extracts exhibited slightly higher activity against C. albicans than against A. niger. Ethanol extract show 6 mm and 8 mm, methanol extract display 6 mm and 9 mm, one more acetone extract show 5 mm both fungi of ZOI against A. niger and C. albicans, respectively, at 1% w/v concentrations **(Table 6)**.

Amphotericin-B showed higher inhibitory activity against A. niger than C. albicans, and its combinations with extracts behave the slightly higher than standard drug. Combinations of Amphotericin-B with acetone extract show 20 mm and 20 mm of ZOI against A. niger and C. albicans, respectively, at 1 %w/v concentrations. Other hand combinations of Amphotericin-B with ethanol and methanol extract show 21 mm and 20 mm of zone of inhibition against A. niger and C. albicans, respectively, at 25 µg/ml concentrations. **(Table 7)**.

The combination of fluconazole with acetone extract exhibited 22 mm and 18 mm, of ZOI against A. niger and C. albicans, respectively at 1% concentrations. Ethanol extract showed 33 mm and 23 mm of zone of inhibition against A. niger and C. albicans, individually and methanol extract exhibited 24 mm and 17 mm, of ZOI against A. niger and C. albicans, respectively at 1% concentrations **(Table 8)**.

With lowering extract concentrations, all of the combinations became less efficient against both of the tested fungi.

Table 6: Antifungal activity of pure extract

Fungus	Acetone Extract				Ethanol Extract				Methanol Extract			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	05	05	04	03	06	05	05	04	06	05	04	04
C. albicans	05	05	04	03	08	06	05	04	09	08	04	04

Table 7: Antifungal activity of extract with Amphotericin-B

Fungus	Acetone Extract + Amphotericin-B				Ethanol Extract + Amphotericin-B				Methanol Extract + Amphotericin-B			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	20	20	19	18	21	20	19	19	21	21	20	20
C. albicans	20	20	19	18	20	20	19	18	20	20	19	18

Table 8: Antifungal activity of extract with Fluconazole

Fungus	Acetone Extract + Fluconazole				Ethanol Extract + Fluconazole				Methanol Extract + Fluconazole			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	22	22	21	20	33	31	30	29	24	23	23	22
C. albicans	18	18	17	17	23	21	20	19	17	17	16	15

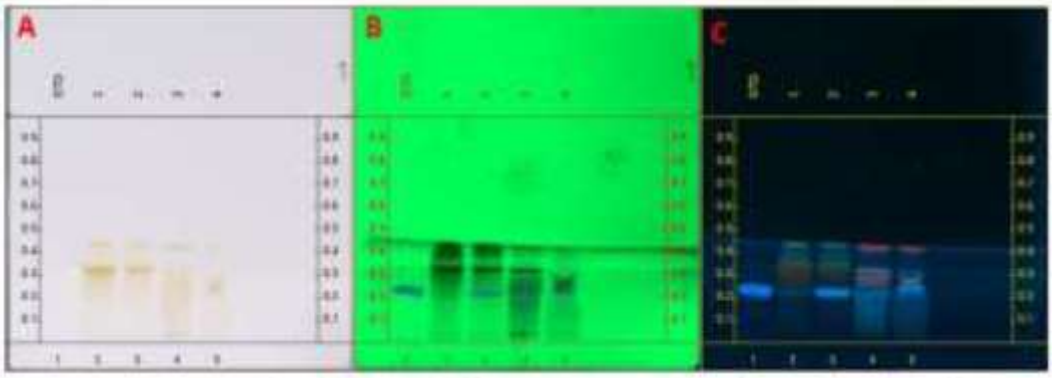

Figure 1: HPTLC of Amoxicillin, extracts and combination at (A) Visible, (B) UV 254 nm, (C) UV 366 nm.

In Figure 1 (A/B/C) represent the same HPTLC experiments result in diff. lights. The first band of Amoxicillin also present at the same Rf value in the third and fifth band which is the combination of Amoxicillin and methanol extract & Amoxicillin and ethanol extract respectively. No any new band is visible and no old band has Disappeared which confirms no any new compound formed by combining antibiotics and extract and so why no further study require for toxicity.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 22 April 2023 Revised: 18 May 2023 Accepted: 23 June 2023

CONCLUSIONS

The result of our study indicated that W. somnifera gave good combination effects with different selected drug. In addition to reducing side effects by lowering antibiotic concentrations, plant extracts can be utilized to increase the antibacterial activity of antibiotics. Following adequate toxicological investigations, combinatorial chemistry can replace the usage of conventional drugs.

Acknowledgements

The authors are thankful to the Gokul Science College, Sidhpur for providing the necessary laboratory and library facility.

Conflict of Interest

The authors confirm that this article's content has no conflict of interest.

REFERENCES

1. Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. Alternative medicine review, 5(4), 334-346.
2. Kulkarni, S. K., & Dhir, A. (2008). Withania somnifera: an Indian ginseng. Progress in neuro-psychopharmacology and biological psychiatry, 32(5), 1093-1105.
3. Archana, R., & Namasivayam, A. (1998). Antistressor effect of Withania somnifera. Journal of ethnopharmacology, 64(1), 91-93.
4. Umadevi, M., Rajeswari, R., Rahale, C. S., Selvavenkadesh, S., Pushpa, R., Kumar, K. S., & Bhowmik, D. (2012). Traditional and medicinal uses of Withania somnifera. The pharma innovation, 1(9, Part A), 102.
5. Dar, N. J., Hamid, A., & Ahmad, M. (2015). Pharmacologic overview of Withania somnifera, the Indian Ginseng. Cellular and molecular life sciences, 72, 4445-4460.
6. Bhattacharya, S. K., Satyan, K. S., & Ghosal, S. (1997). Antioxidant activity of glycowithanolides from Withania somnifera. Indian journal of experimental biology, 35(3), 236-239.
7. Bhattacharya, S. K., Goel, R. K., Kaur, R., & Ghosal, S. (1987). Anti-stress activity of sitoindosides VII and VIII, new acylsterylglucosides from Withania somnifera. Phytotherapy research, 1(1), 32-37.
8. Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian journal of chemistry, 10, S1193-S1199.
9. Rai, M., Jogee, P. S., Agarkar, G., & Santos, C. A. D. (2016). Anticancer activities of Withania somnifera: Current research, formulations, and future perspectives. Pharmaceutical biology, 54(2), 189-197.
10. Sharifi-Rad, J., Quispe, C., Ayatollahi, S. A., Kobarfard, F., Staniak, M., Stępień, A., ... & Cho, W. C. (2021). Chemical Composition, Biological Activity, and Health-Promoting Effects of Withania somnifera for Pharma-Food Industry Applications. Journal of Food quality, 2021(1), 8985179.
11. Tewari, D., Chander, V., Dhyani, A., Sahu, S., Gupta, P., Patni, P., ... & Bishayee, A. (2022). Withania somnifera (L.) Dunal: Phytochemistry, structure-activity relationship, and anticancer potential. Phytomedicine, 98, 153949.
12. Munir, N., Mahmood, Z., Shahid, M., Afzal, M. N., Jahangir, M., Ali Shah, S. M., ... & Yousaf, F. (2022). Withania somnifera chemical constituents' in vitro antioxidant potential and their response on spermatozoa parameters. Dose-Response, 20(1), 15593258221074936.
13. Kumar, V., Dey, A., Hadimani, M. B., Marcovic, T., & Emerald, M. (2015). Chemistry and pharmacology of Withania somnifera: An update. CellMed, 5(1), 1-1.
14. Singh, G., & Kumar, P. (2014). Antibacterial activity of flavonoids of Withania somnifera L. International Journal of Green Pharmacy (IJGP), 8(2).
15. Khanna, P. K., Kumar, A., Ahuja, A., & Kaul, M. K. (2007). Fatty Acid Composition of Seed Oil of Withania somnifera. Physiol. Mol. Biol. Plants, 13(1), 73-76.
16. Kumar, B., Bajpai, V., Gond, V., Tiwari, S., & Madhusudanan, K. P. (2021). Phytochemistry of Withania somnifera. CRC Press.
17. Owais, M., Sharad, K. S., Shehbaz, A., & Saleemuddin, M. (2005). Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 12(3), 229-235.
18. A Dar, P., R Singh, L., A Kamal, M., & A Dar, T. (2016). Unique medicinal properties of Withania somnifera: Phytochemical constituents and protein component. Current pharmaceutical design, 22(5), 535-540.
19. Golanowska, M., Kielar, J., & Lojkowska, E. (2017). The effect of temperature on the phenotypic features and the maceration ability of Dickeya solani strains isolated in Finland, Israel and Poland. European Journal of Plant Pathology, 147, 803-817.
20. Golanowska, M., & Lojkowska, E. (2016). A review on Dickeya solani, a new pathogenic bacterium causing loss in potato yield in Europe. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology, 97(2).

21. van der Wolf, J. M., Cahill, G., Van Gijsegem, F., Helias, V., Humphris, S., Li, X., ... & Pritchard, L. (2021). Isolation, detection and characterization of Pectobacterium and Dickeya Species. Plant Diseases Caused by Dickeya and Pectobacterium Species, 149-173.
22. Golanowska, M., & Lojkowska, E. (2016). A review on Dickeya solani, a new pathogenic bacterium causing loss in potato yield in Europe. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology, 97(2).
23. Burra, D. D., Mühlenbock, P., & Andreasson, E. (2015). Salicylic and jasmonic acid pathways are necessary for defence against D ickeya solani as revealed by a novel method for blackleg disease screening of in vitro grown potato. Plant Biology, 17(5), 1030-1038.
24. Khan, M. A., Zhao, Y., & Korban, S. S. (2012). Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen Erwinia amylovora, causal agent of fire blight disease in Rosaceae. Plant Molecular Biology Reporter, 30, 247-260.