

Different Formulations of *Curcuma Longa* and *Withania Somnifera* Possess Anti-Microbial Activities *In-Vitro*

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Abstract

Background: The world is facing a big challenge of antimicrobial resistance (AMR). Herbs have tremendous potential of antimicrobial activities. This study was aimed to find out the antimicrobial activity of aqueous ethanol extracts of *Curcuma longa* (CL) and *Withania somnifera* (WS) individually and in different ratios of formulations.

Materials and methods: Herbs CL and WS were extracted in a predefined ratio of aqueous ethanol medium, condensed and the formulations were prepared in 25:75, 50:50 and 75:25 ratios respectively. Culture mediums of *Bacillus subtilis* and *Serratia marcescens* were prepared. A disc with the samples CL, WS, 25:75, 50:50 and 75:25 were placed in the cultured plate. The inhibition of microbial growth around the discs indicated the zone of inhibition. The presence or absence zone of inhibition was noted and its diameter was calculated.

Results: CL did not show any antimicrobial activity against *Bacillus subtilis* individually. However, when combined with WS, it showed a significant effect. WS showed maximum effect against *Bacillus subtilis*, followed by 25:75, 50:50 and 75:25. In case of *Serratia marcescens*, 25:75 showed maximum effect, followed by 75:25. Other extracts showed almost similar effect.

Conclusion: CL and WS and their formulations have significant antimicrobial effect against *Serratia marcescens*. WS alone and in formulation with CL showed antimicrobial effect against *Bacillus subtilis*, but not CL. Formulation 25:75 showed better effect than other samples.

Keywords: *Curcuma longa*, *Withania somnifera*, antimicrobial, *Bacillus subtilis*, *Serratia marcescens*

INTRODUCTION

Antimicrobial resistance (AMR) is becoming a big challenge globally. AMR is declared by World Health Organization as one of the top ten threats to public health facing humanity worldwide [1]. Antimicrobials have been misused and overused in many countries, which lead to drug-resistant pathogens. It is very challenging in both developed and developing worlds to control. Especially in developing countries where sanitary measures and preventive aspects are insufficient, some microbes become resistant to antibiotics. Expenditure due to AMR is becoming higher and higher. Because it not only results in death and disability but also in prolonged hospital stays, lengthy treatment, costlier medication, etc. It also makes many modern medical procedures like surgery and chemotherapy risky. However, many medicinal plants have shown antimicrobial activities. For instance, *Curcuma longa* (CL) and *Withania somnifera* (WS) are among the most commonly used medicinal plants in Indian medicine for many disorders for centuries. Recently some of the extracts of these plants have been reported to possess anti-microbial potential [2-7]. But further exploration is needed on these plant extracts.

CL, commonly known as turmeric or Haldi in Hindi, has been one of the most used spices in India for many centuries. WS, commonly known as Ashwagandha and also called "Indian Winter cherry" or "Indian Ginseng," is also used for millennia in Indian medicine for the treatment of various disease conditions and as a special tonic due to its health benefits [8-11]. CL contains 3-5% volatile oils like zingiberan, turmerone and other compounds like curcumin, bitter principles, and resins. The active phytoconstituents present in turmeric are collectively termed curcuminoids which mainly include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemthoxy curcumin. It is well documented for antitussive, antifungal, cholesterol-lowering, anticancer, anti-rheumatic and other properties of its different extracts [12-17]. WS has over 12 alkaloids, 40 withanolides and several sitoindosides. The main constituents include withanine, withananine, nicotine, somnifer, somniferincine, along with other alkaloids, sugars, β -sitosterol, etc. It is reported for diverse activities like an adaptogenic, aphrodisiac, brain tonic, diuretic, antihelminthic, astringent, thermogenic and stimulant, anti-stress, anti-inflammatory, anti-ulcer, anti-aging and anti-rheumatism properties. WS is very frequently used to restore vitality

after overwork or nervous exhaustion, and reported for beneficial effect for the debility associated with long-term stress. Due to high iron content, it is recommended in anaemia and related weakness [18-25].

Despite various studies reporting that these plants possess antimicrobial effects in different extract forms, none of them has reported the effects of aqueous ethanolic extracts for their antimicrobial activity. Hence, we envisioned undertaking this study to determine the antimicrobial effects of the aqueous-ethanolic extracts of CL and WS against *Serratia marcescens* (SM) and *Bacillus subtilis* (BS).

MATERIALS AND METHODS

Aqueous ethanol herbal extract preparation

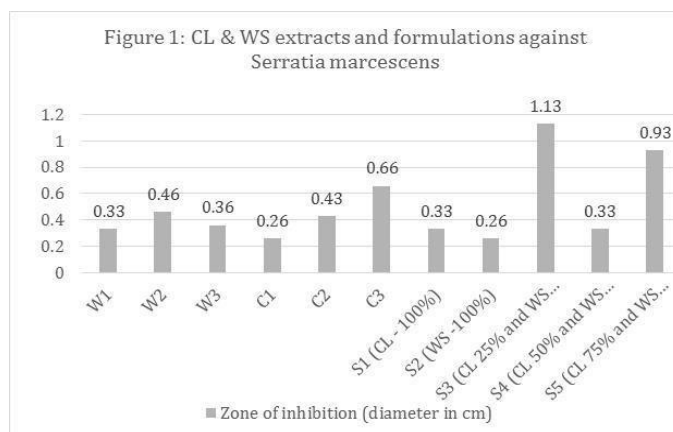
Herbs have been procured from the herbal garden of Dr. Willmar Schwabe India Pvt. Ltd. (Schwabe India). They were identified by the botanist at Schwabe India and sent to the National Institute of Science Communication and Information Resources for confirmation, which was duly confirmed. A specimen herb was submitted at the herbarium of Schwabe India and the specimen number was obtained. A comparative study of High-performance thin-layer chromatography (HPTLC) and ultraviolet (UV) spectroscopy was also performed with control samples to ensure their standards. CL and WS were extracted using water and ethanol. 100 g of CL fresh rhizome was extracted with 400 ml of water and 635 ml of ethanol using the cold percolation method. The extract was then filtered and the resulting liquid was concentrated under reduced pressure at 45 °C in a rotary evaporator. This extract was then kept in the incubator at 45 °C for 3 days to remove the ethanol residue yielding the crude rhizome extract. This extract was then diluted with distilled water at a ratio of 5:1 to prepare the extract stock solution (200 mg/mL). 100 g of WS fresh rhizome was extracted with 250 ml of purified water and 800 ml of ethanol using the methods as above for CL. Water ethanol ratio was defined as per Homoeopathic Pharmacopeia of India [26]. Later, extracts of CL and WS were combined in the ratios of 25:75, 50:50 and 75:25 respectively. These formulations and individual extracts were compared with the pure compounds of Curcumin and Withanoloids in different concentrations of 150 mg, 250 mg and 350 mg and coded as C1 or W1, C2 or W2 and C3 or W3 respectively.

Culture preparation and antimicrobial activity

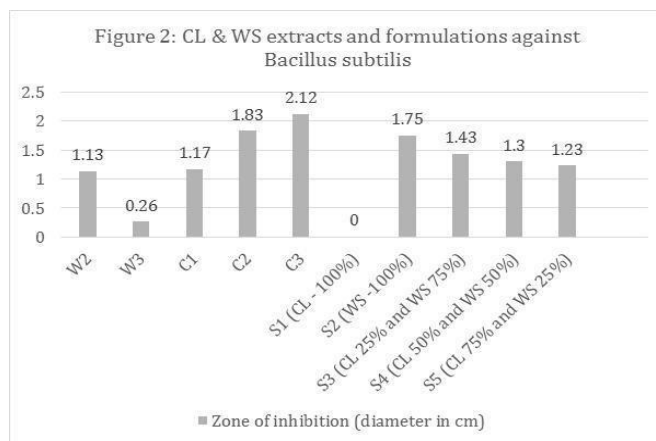
Nutrient broth for bacteria was prepared using 2.6g of nutrient broth in 200 ml distilled water. The broth was divided into four falcon tubes and inoculated with each of the microbes using a sterile inoculating loop (*Bacillus subtilis* and *Serratia marcescens*). Potato Dextrose broth for fungi was prepared using 2.4g of nutrient broth in 100ml distilled water. This was divided into two falcon tubes, and *Piriformospora indica* was inoculated in one of them using a sterile inoculating loop. The bacteria were incubated at 37 degrees Celsius for 24 hours. Nutrient agar media was prepared for bacteria using 5.6g of nutrient agar media in 200ml of distilled water. Potato Dextrose Agar was prepared for fungi using 3.9g of potato dextrose agar in 100 ml of distilled water. Whatman blotting paper was used to prepare the discs for disk diffusion. They were then placed in a jam bottle. The media, discs and glass plates were then autoclaved at 121 degrees Celsius for 15 minutes. The spread plates were prepared using a glass spreader. The discs, impregnated with the plant extract samples, were placed on the spread plate of each of the microorganisms. The diameter of the disc was 0.5 cm. The bacterial plates were incubated at 37 degree Celsius for 24 hours and the fungal plates were incubated at 37 degree Celsius for ten days. The inhibition of microbial growth around the discs indicated the zone of inhibition. The presence or absence zone of inhibition was noted and its diameter was calculated.

RESULTS AND DISCUSSION

In case of *Serratia marcescens*, 25:75 showed maximum effect, followed by 75:25 and the curcumin 350 mg. Other extracts have shown an almost similar effect. Formulated extracts were more effective than even the pure compounds of Curcumin and Withanoloids.



Surprisingly, aqueous ethanol extract of Curcumin has not shown any antimicrobial activity against *Bacillus subtilis* individually.



However, when combined with WS, it showed a significant effect. WS showed maximum effect against *Bacillus subtilis*, followed by 25:75, 50:50 and 75:25. But the Curcumin pure compound showed more effect than the aqueous ethanol extracts. Niamsa *et al* [2], Gupta *et al* [3], etc. reported CL aqueous extract showing antimicrobial effect against *Escherichia coli*, *Staphylococcus aureus*, *Klebsilla pneumoniae* and *Staphylococcus epidermidis*. However, in this study it did not show any effect against *Bacillus subtilis*. But it showed significant effect against *Serratia marcescens*. On similar lines, Mehrotra *et al.* [27] and Singariya *et al.* [28] reported that of WL. Our study confirms the same. Formulation 25:75 showed maximum effect against *Serratia marcescens*, followed by 75:25.

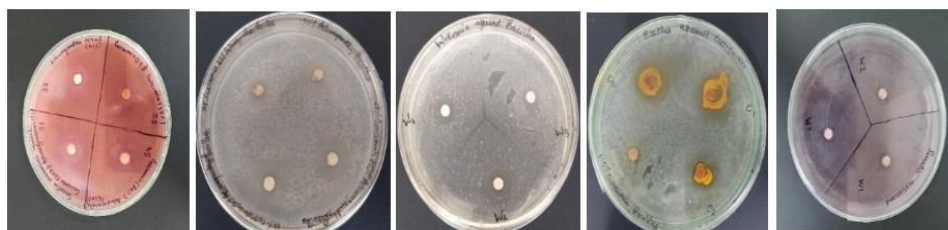


Figure 3 (A): WS 100%, CL WS 25:75, 50:50 & 75:25 against SM; (B) WS 100%, CL WS 25:75, 50:50 & 75:25 against BS; (C) WS against BS; (D) CL against BS; (E) WS against SM

CONCLUSION

This study shows that aqueous ethanol extracts of CL, WS and their formulations possess antimicrobial activity against *Serratia marcescens*. WS alone and in formulation with CL showed antimicrobial effect against *Bacillus subtilis* but CL individually did not show such activity. Among the formulations, 25:75 showed a better effect than other samples.

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CONFLICT OF INTEREST

None

AUTHOR'S CONTRIBUTION

HK conceptualized the study, RV conducted the experiments, RV prepared preliminary manuscript and HK contributed in finalizing the manuscript.

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None

Ethics Statement

NA

Informed Consent

NA

Data Availability

NA

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