Phytology

Comparative study of antimicrobial activity of *in-vivo* and *in-vitro* generated *Withania somnifera* leaf extracts against some known human pathogenic bacteria

Suryakanta Bahira[1, 2*], Tapaswini Moharana[3], Tejashweta Samal[1, 4], Sangram Keshari Samal[1, 5], Ghanishtha Prusty[1, 6]

[1] Ph.D Scholar, School of Sciences, GIET University, Gunupur, Rayagada, Odisha, India

[2] Department of Botany, Palsama Science Degree College, Deogarh, Odisha, India

[3] Department of Botany, Brahmani Degree College, Kendrapara, Odisha, India

[4] Department of Biology, SCITM, Koraput, Odisha, India

[5] Department of Zoology, S.C.S Autonomous College, Puri, Odisha, India

[6] Department of Zoology, School of Sciences, GIET University, Gunupur, Rayagada, Odisha, India

Correspondence Author: Suryakanta Bahira

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Abstract

Withania somnifera is one of the most valuable medicinal plants used in human and veterinary disorders. The present work was proposed to consider the possibility of the antimicrobial effect of in-vivo and in-vitro generated leaf extracts of *W. somnifera* against some known bacterial strains and the chance of utilizing the constituents present there for the preparation of pharmaceutical products. This is achieved through the zone of inhibition studies and minimum inhibitory concentrations. For this work, four known bacterial strains were used. Among them, two were Gram-positive namely *Streptococcus pyrogens* and *Streptococcus mutans,* and two were Gram-negative, namely *Salmonella typhimurium and Vibrio cholerae*. The inhibition zones of different plant extracts were then compared with standard antibiotics like Neomycin, Kanamycin, and Gentamycin. In comparison to ethanolic extract, methanolic plant extract produces better results. Again, in-vivo generated plant extracts are comparatively better than in-vitro generated plant extracts. The methanolic extract shows maximum antimicrobial activity against *Streptococcus mutans,* followed by *Salmonella typhimurium*. For all the plant extracts, *Vibrio cholerae* shows the least inhibition zone. These opportunities can build up innovative knowledge in the research of medicinal products.

Keywords: *Withania somnifera*, antimicrobial activity, leaf extract, in-vivo, in-vitro, inhibition zone, antibiotic, medicinal product

Introduction

Withania somnifera is a small-sized, erect, greyish, perineal woody shrub of the family Solanaceae. It is widely known in India by different vernacular names, like Indian Ginseng (English), Asgandha (Hindi), Asuragandhi (Tamil), Asvagandhi (Telugu), Asgand (Punjabi), Ashwagandha (Bengali, Oriya, and Sanskrit). There are about 23 species known to be widely distributed in the world, out of which only two species, W.coagulans (L.) Dunal and *W.somnifera* (L.) Dunal are medicinally significant [1, 2]. The plants are commonly used in Ayurvedic medicine in India and their therapeutic importance is mentioned in the Charaka Samhita [3]. The roots of this plant are the source of drugs and have got a wide range of applications in the treatment of hiccup, female disorders, and cough, rheumatism, tuberculosis, and exhibit excellent anti-tumour and antibacterial activities [4, 5]. Modern herbalists categorize Ashwagandha as an adaptogen, which increases the body's ability to withstand stress of all kinds. It shows helpful effects on the endocrine, cardiac, and central nervous systems. The predominant biochemical ingredients of Ashwagandha root are steroidal alkaloids and steroidal lactones in a category of parts referred to as Withanolides [6]. According to Singh *et al*. (2012), the alkaloid extracts from various parts of *Withania somnifera* (root, stem, leaf, and fruit) have antimicrobial activity against *Enterobacter aerogens, Bacillus subtilis*, *Klebsiella pnemoniae*, *Agrobacterium tumefaciesns*, and *Raoultella planticola* [7]. The results show that the stem alkaloid extract of *Withania somnifera* has the highest antimicrobial activity against *Enterobacter aerogens*, while the root extract has significant activity against all test bacteria. The leaf extracts of *Withania somnifera* were tested against some human and plant pathogens like *Proteus mirabilis*, *Klebsiella pnemoniae, Agrobacterium tumefaciens,* and *Aspergillus niger* [8]. In 2011, Kumar *et al*. screened the leaf and root extract of *Withania somnifera* against some human pathogenic bacteria (*Escherichia coli*, *Bacillus subtilis,* and *Shigella sp*.) and fungi (*Aspergillus niger* and *Trichophyton rubrum*). The leaf sample shows higher antimicrobial activity than the root sample [9]. Antimicrobial screening of *in-vitro* leaves of *Withania somnifera* was carried out by Sunderam *et al*., in 2011. The different solvents used for this extraction were ethanol, ethyl acetate, dichloromethane, and hexane. Antimicrobial activity was determined against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Bacillus subtilis*. Ethyl acetate extract of *Withania somnifera* has shown significant activity against

Staphylococcus aureus and *Bacillus subtilis* [10].

2. Materials and Methods

For the preparation of plant extracts, both in-vivo and in-vitro grown healthy plant leaves were collected, and the percolation method was used to obtain the extracts of these plant leaves. The leaves were dried at room temperature under shade conditions for 15 days. After that, the leaves were powdered in a grinder and sieved separately. Five grams of the material were soaked in 40 ml of ethanol, and methanol respectively, in two different conical flasks and were left overnight in a rotary shaker. After a day, the residues were filtered separately using eight layers of clean cheesecloth. Then the extracts were transferred to a beaker and dried at room temperature for 24 hours. Then these extracts were dissolved in an appropriate volume of dimethyl sulfoxide (DMSO) to get a stock concentration of 50 mg/ml. The samples were then stored at 4^0c in a refrigerator for future use.

Screening of antimicrobial activity

For conducting this experiment, four bacterial strains were used, i.e., two were Gram-positive namely *Streptococcus pyrogens* and *Streptococcus mutans* and two were Gram-negative namely *Vibrio cholerae* and *Salmonella typhimurium*. Nutrient agar is considered a suitable medium for bacterial growth. The antimicrobial activity of the extracts against these test pathogens was achieved by using the Agar cup plate method [11]. Nutrient agar plates were prepared, and the microbial strains were seeded over the plates by using a sterile glass spreader inside a laminar airflow chamber. Wells of 0.8 cm diameter were made on the agar plate by using a sterile cork borer and filled with 100µl of plant extracts inside a laminar airflow chamber. The cultured plates were then incubated at 37° C for 24 hours. After the incubation period, the inhibition zones around each well were observed, which confirmed the antimicrobial activity of the respective leaf extract. The same procedure was followed for each leaf extract and bacterial strain. The clear zone formed was measured and the average diameter of the inhibition zone was taken for evaluation of the antimicrobial effect of the extracts. Inhibition zones of plant extracts were compared with standard antibiotics like Kanamycin, Neomycin, and Gentamycin.

3. Results and Discussion

The leaf extracts of *Withania somnifera* exhibit promising results against all bacterial strains tested (Table-1, Figure-1 and 2). Inhibition zones ranged between 19 and 34.33 mm. In contrast to ethanol extract, methanol extract shows a significant result in both cases of in-vivo and in-vitro grown plants (Table-1, figure-3 and 4). For all the plant extracts, *Vibrio cholerae* shows the least inhibition zone. *Streptococcus mutans* shows the highest zone of inhibition for both ethanolic and methanolic extracts of in-vivo grown plants, i.e.,32.33±1.14 and 34.33±0.56. For in-vitro grown plants, *Salmonella typhimurium* shows the highest zone 21.4±0.58 and 25.13±0.52. Both ethanol and methanol extracts show an inhibitory effect on microbial growth against all the bacterial strains. The test microbes were found to be sensitive to all the leaf extracts used in the experiment.

Table 1: Antimicrobial effect of different solvent extracts of both in-vivo and in-vitro grown *Withania somnifera* against different microbial strain

Bacterial strain	Diameter of zone of inhibition (in mm)			
	Solvent extract			
	Ethanol		Methanol	
	in-vivo	*in-vitro*	*in-vivo*	*in-vitro*
Streptococcus mutans	32.33±1.14	19.65±0.15	34.33±0.56	24.12±0.40
Streptococcus pyrogens	30.66±0.56	19.55±85	32.33±1.14	23.98±0.82
Salmonella typhimurium	30.83±0.55	21.4±0.58	30.33±0.56	25.13±0.52
Vibrio cholera	29.31±1.5	19 ± 0.52	30.12 ±1.11	21.33±0.53

Table 2: Comparison of zone of Inhibition of plant extracts with Standard Antibiotics

Bacterial strain	Diameter of zone of inhibition (in mm)		
	Kanamycin	Neomycin	Gentamycin
Streptococcus mutans	17.33±0.58	22.67±0.58	26±12
Streptococcus pyrogens	17.67±0.58	21.67±0.58	28.33±0.58
Salmonella typhimurium	15.67±0.58	21.33±0.58	26.33±0.58
Vibrio cholera	16.33±1.53	21.33±0.58	26.67±0.58

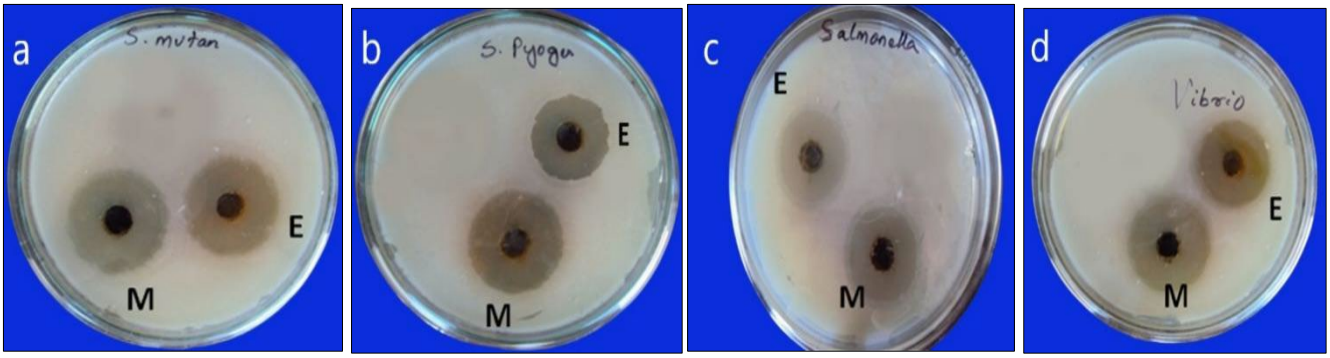

Fig 1: Inhibitory zones shown by in-vivo grown leaf extracts of *Withania somnifera* against (a. *Streptococcus mutans* b. *Streptococcus pyrogens* c. *Salmonella typhimurium* d. *Vibrio cholera*)

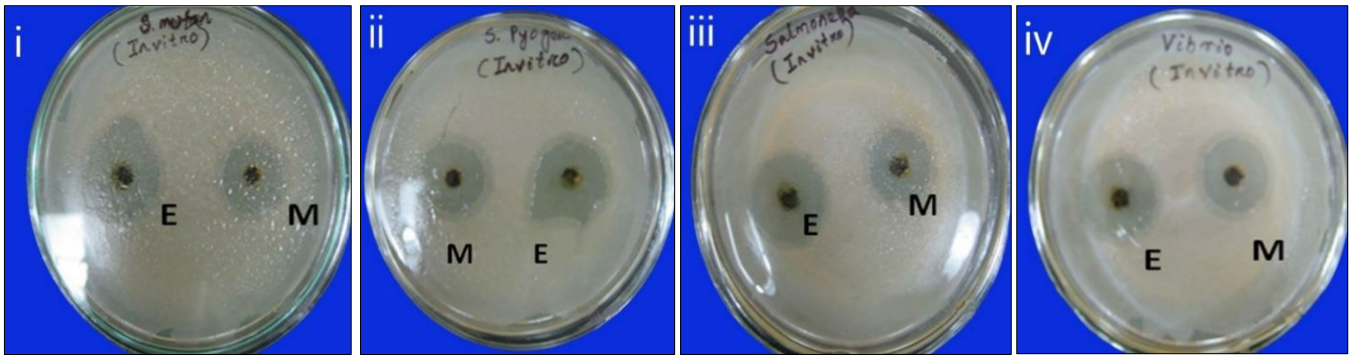

Fig 2: Inhibitory zones shown by in-vitro grown leaf extracts of *Withania somnifera against* (i. *Streptococcus mutans* ii. *Streptococcus pyrogens* iii. *Salmonella typhimurium* iv. *Vibrio cholera*)

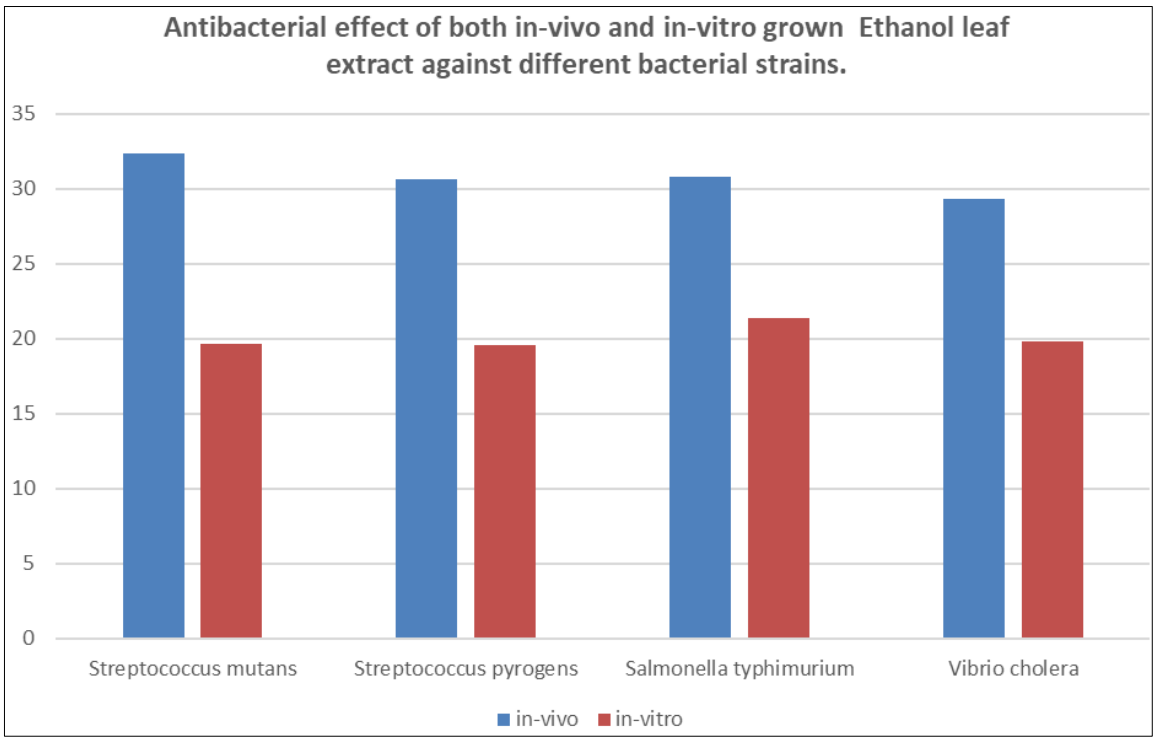

Fig 3: Antibacterial effect of both in-vivo and in-vitro grown ethanolic leaf extract against different bacterial strains

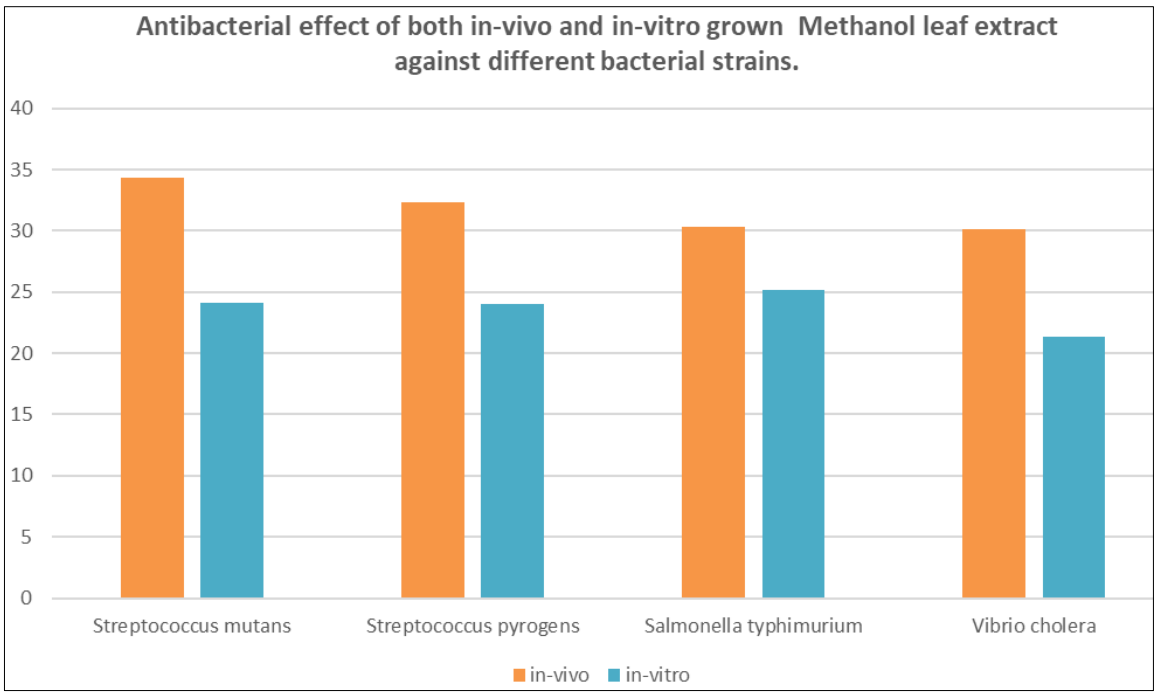

Fig 4: Antibacterial effect of both in-vivo and in-vitro grown methanolic leaf extract against different bacterial strains

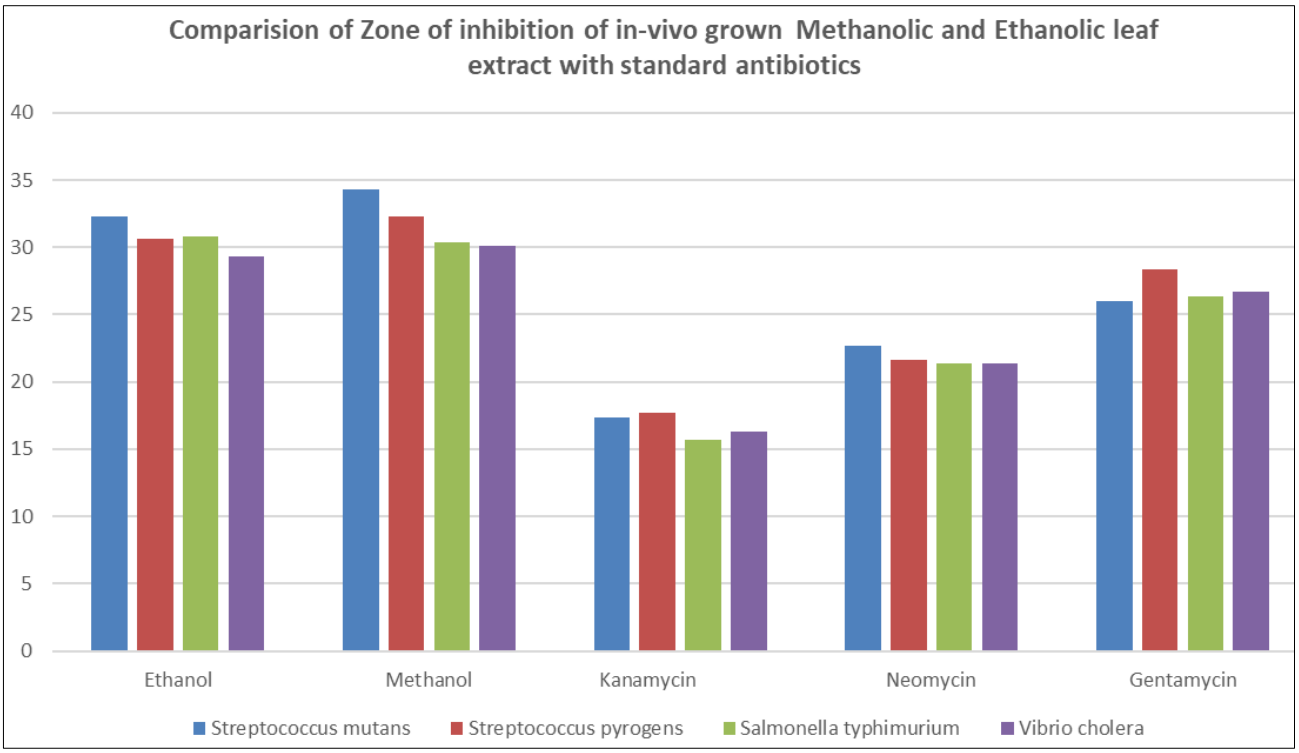

Fig 5: Comparison of Zone of inhibition of in-vivo grown Methanolic and Ethanolic leaf extract with standard antibiotics

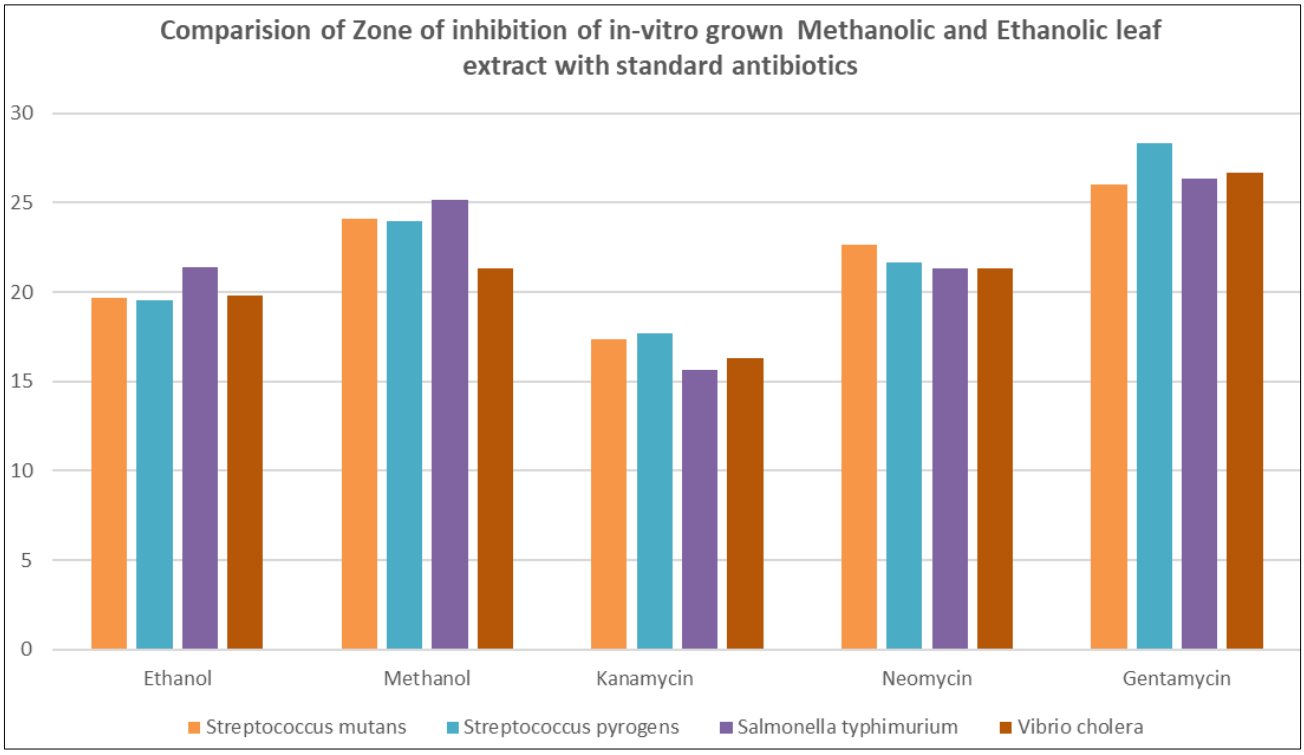

Fig 6: Comparison of Zone of inhibition of in-vitro grown Methanolic and Ethanolic leaf extract with standard antibiotics

4. Conclusion

The present work was carried out to evaluate the antimicrobial activity of in-vivo and in-vitro generated *Withania somnifera* leaves against some known human pathogenic bacteria. Ethanol and methanol extracts of both in-vivo and in-vitro grown leaves were prepared. The antimicrobial activity of these extracts was evaluated by the Agar cup method. All the extracts show inhibitory effects against all the bacterial strains, but the methanol extract reveals better results comparatively than the ethanol extract. Again, in-vivo collected plant extracts were comparatively better than in-vitro generated plant extracts. Inhibition zones were comparable to those of standard antibiotics. The methanol extract shows maximum antimicrobial activity against Streptococcus *mutans,* followed by *Salmonella typhimurium*.*W. somnifera*is one of the most valuable medicinal plants used in human and veterinary disorders. Such experiments can validate the use of plant extracts for specific pathogens or diseases. However, the biochemical composition of the extracts and the active ingredients present in these extracts need to be examined before proposing their use in pharmaceuticals. This work could not be done within the limited scope of the laboratory and

within a stipulated period. Those aspects need to be further studied to derive the full potential of the plants.

5. References

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