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Study on antimicrobial activity of leaf extract of *Withania somnifera* L. Dunal against clinical pathogens

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Abstract

Herbal medicine is one of the most remarkable uses of plant based biodiversity. Medicinal plant plays a key role in world healthcare system. In traditional system of medicine different part like stem, leaf, flower, root, seeds of *Withania somnifera* L.Dunal (known as Ashwagandha) are used. The present study was undertaken to evaluate the antimicrobial activity of different solvent extracts of *Withania somnifera* leaf against clinical pathogen. Leaf extract obtained with ethanol and methanol was evaluated by disc diffusion method and zone of inhibition was measured. The minimum inhibitory concentration (MIC) of the leaf extracts for antibacterial activity measured was 2-5mg/ml and ethanolic extract produced maximum zone of inhibition 22-24mm on all the microorganisms. From this solvents showed higher activity in the order of ethanol> methanol. The plant leaf extract of *Withania somnifera* showed higher activity for different clinical pathogens with significant antimicrobial activity on *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris* and *Pseudomonas aeuroginosa.* All these finding support the antimicrobial use of different solvent extract of *Withania somnifera* leaf.

Keywords: *Withania somnifera*, antimicrobial activity, clinical pathogen.

Introduction

Herbal medicine is one of the most remarkable uses of plant based biodiversity. Nature has provided a complete storehouse of remedies to cure human aliments. About 80 percent of the world's population depends fully or partially on traditional medicine for primary health care needs (Kunwar & Adhikari, 2005). Medicinal plants play a key role in world health care system (Bajaj & Williams, 1995). Nature has bestowed on us a very rich botanical wealth and a large number of diverse types grow in different parts of country (Joshi *et al*., 2011). In traditional system of medicine different parts like stem, leaf, flower, root, seeds of *Withania somnifera* are used. Ashwagandha is one of the best Ayurvedic herbs and holds a place in the Ayurvedic traditions similar to Ginseng in Chinese therapies. It has been often referred to as the "IndianGinseng". It has been used for thousands of years as a popular remedy in Ayurvedic system for many conditions. The objective of the present study was to compare the antimicrobial activity of *Withania somnifera* leaves extract against different clinical pathogens.

Plant Descriptions:

Withania somnifera L. Dunal (Ashwagandha), popularly known as Indian ginseng and Winter Cherry, is one of the most reputed medicinal herbs that forms an essential constituent of over 100 traditional medicine formulations. Several other species in the genus *Withania* are morphologically similar. It is known for its wide range of therapeutic uses in ayurvedic and other traditional systems of medicine.

More than 91 pharmaceutical products are produced from this plant .The species name *somnifera* means sleep-inducing in Latin (Stearn, 1995).

Hindi : Asgandh, Punir
Sanskrit: Ashwagandha, Turangi-gandha.
English : Winter cherry.

Materials and Methods

Collection of plant material

Fresh leaves of the plant *Withania somnifera* free from disease were collected from Botanical garden of Govt. E. R. Rao Science College, Bilaspur (C.G.). The leaves were washed thoroughly 2-3 times with running water and one with sterile distilled water. It was then air dried on sterile blotter and shade.

Solvent extraction

Thoroughly washed dried leaves of this plant were dried in shade for 4-5 days and then powdered with the help of blender. Twenty five grams (25g) of Ashwagandha leaves dried powder was allowed to soaked for 24 hours in 100 ml of different extractants (ethanol, methanol) and for better extraction, incubated in orbital shaker for 48 hrs. The solvent extracts were filtered concentrated under reduced pressure and stored at 5^0C in airtight bottle for further use.

Growth and maintenance of test microorganism for antimicrobial activity

Bacterial cultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris* and *Pseudomonas aeuroginosa* were obtained from department of Biotechnology, Govt. E. R. Rao PG Science College, Bilaspur (C.G.). They were used for antimicrobial test organism. The bacterial cultures were maintained on nutrient broth (NB) at 37^0C.

Disc diffusion method

Antimicrobial activity of the leave extracts was tested by using the disc diffusion method (Mahmood et al., 2008). Sterile nutrient agar plates were prepared and bacterial strains inoculated by spread plate methods in aseptic conditions. The sterile filter paper disc of 5mm diameter (Whatman no. 1 filter paper) was prepared. Also methanolic leaves extract were prepared in various concentrations of 5µl, 25µl, 50µl and 100µl and experiment disc were dipped for before putting on medium. The sterile impregnated disc with leaves extract were placed on the agar surface with framed forceps and gently pressed. Filter paper discs soaked in solvent were used for negative controls. All the plates were incubated at 37^0C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured by antibiotic zone scale meter.

Results

Result obtained in the present study revealed that tested medicinal plant extracts with ethanol and methanol posses potential antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris* and *Pseudomonas aeuroginosa*. When tested by the disc diffusion method the ethanol leaf extract of *Withania somnifera* showed significant higher activity against clinical pathogen around maximum range between 22-24 mm (Table 1.2; Figure.1) comparatively with methanol extracts which showed antimicrobial activity range between 20-22mm with all tested bacterial culture (Table 1.1). As the concentration of leaf extracts of plant increased from 5 < 25 < 50 < 100, the zone of inhibition with different microbial cultures were also increased and maximum zone of inhibition from 22-24 were reported in 100 µl/ml concentration of leaf extract with ethanol and methanol both where as the least values were obtained with the 5µl/ml concentration of leaf extract that was between in ranges from 2-4mm.

Table 1.1 Inhibitory zones of methanolic extracted *Withania somnifera* leaves against different clinical pathogens.

Sample	Clinical Pathogens	Concentration (µg/ml)			
		5	25	50	100
		Zone of inhibition (in mm)			
Leaf Extract	*Staphylococcus aureus*	3	7	13	21
	Bacillus subtilis	2	4	11	20
	Proteus vulgaris	3	6	13	22
	Pseudomonas aeuroginosa	2	7	12	20

Table 1.2 Inhibitory zones of ethanolic extracted *Withania somnifera* leaves against different clinical pathogens.

Sample	Clinical Pathogens	Concentration (µg/ml)			
		5	25	50	100
		Zone of inhibition (in mm)			
Leaf Extract	*Staphylococcus aureus*	4	7	14	20
	Bacillus subtilis	3	7	13	24
	Proteus vulgaris	3	6	12	22
	Pseudomonas aeuroginosa	4	7	13	20

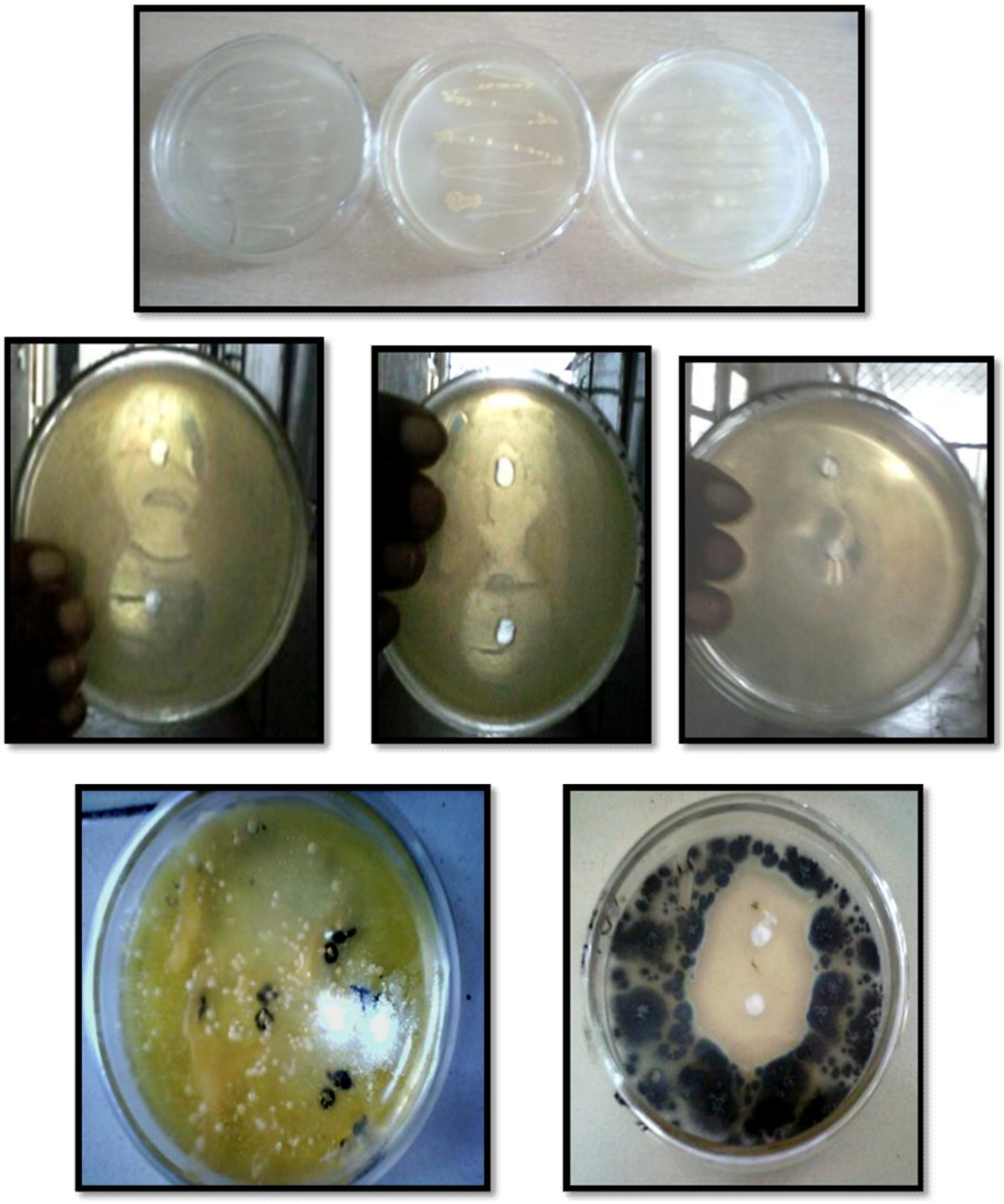

Figure 1. Inhibitory zones of ethanolic extracted *Withania somnifera* leaves against different clinical pathogens.

Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *etal*., 1998). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic and anti-inflammatory properties of plants. (Samy and Ignacimuthu, 2000; Palombo *etal*.,2001; Kumaraswamy *etal*.,2002; Stepanovic *etal*.,2003; Behra and Misra 2005; Bylka *etal*., 2004; Govindarajan *etal*.,2006; Kambizi and Afolayan 2008). However not many reports are available on the exploitation of antibacterial property of plants for developing commercial formulations for application in crop protection.

In the present study the methanolic leaf extracts of *Withania somnifera* showed the activity against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeuroginosa* and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. Further monomeric glycoprotein namely *Withania somnifera* of (*Withania somnifera* glycoprotein) isolated from *Withania somnifera* root tubers revealed antimicrobial activity against few bacterial and phytopathogenic fungi by different workers (Girish *et al*., 2006).

Conclusion

Chhattisgarh is a rich source of biodiversity and many medicinal plants are found here. *Withania somnifera* have lots of medicinal properties so forever it is used to cure different internal and external diseases. All the above findings support the traditional knowledge of local healer and it is a preliminary, scientific validation for the use of these plants for antibacterial activity to promote proper conservations and sustainable use of such plant resources.

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