ANTIMICROBIAL ACTIVITY *WITHANIA SOMNIFERA* (ASHWAGANDHA) BY USING LEAF EXTRACT AGAINST DIFFERENT BACTERIA

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ABSTRACT

The Antimicrobial resistance methodologies and the discovery of novel antimicrobials from plants and other natural sources have been widely used. The genus Withania belong to the soloanaceae family, which comprises 84 genera more than 3,000 species spread around the world.Withania somnifera dunal (WS), commonly known as Ashwagandha in India, belong to the family solanaceae. It is extensively used in most of the Indian herbal pharmaceutical and nutraceuticals. Many plants were used in traditional medicine in many countries. The antimicrobial potential of some solvent extracts of Withania somnifera leaves against different strains of bacteria was investigated. The extensively used of Ashwagandha as a traditional medicine for the treatment of various diseases and observed biological activities were due to secondary metabolites found in the methanolic extract. The present study was aimed to investigate antibacterial activity of methanol extracts of Withania somnifera leaves against parthogenic bacteria. The leaves of Withania somnifera species were collected from town Muradnagar, Ghaziabad, extracted with methanol. Antibacterial activity of crude extracts of Withania somnifera was determined by agar disc diffusion method against gram positive, gram negative bacterial. All tested plant extracts showed varying zones of inhibition against bacteria tested. The highest zone of inhibition of 17mm diameter was noted in Bacillus subtilis with methanol extract while the lowest 7mm zone of inhibition was reported in Escherichia coli. The result obtained exhibit that this plant has good medicinal potential, and it need further phytochemical exploitation to isolate phytochemical constituents having antibacterial activities.

KEY WORDS:-*Withania somnifera, Antibacterial, Pharmaceutical, Ashwagandha.*

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1. INTRODUCTION

The use of herbs as antimicrobial has played an important role in almost all cultures around the world, including Asia, Africa, Europe and America. Among these Solanaceae, which is a numerous family, there are 2300 species(Nabeel Al-Ani *et al*.2013). Over 400 herbs worldwide have been recognized as beneficial in treating diabetes. Such a plant that is known to possess diverse biological properties is *Withania somnifera* dunal known as Ashwagandha in Ayurveda or Indian ginseng (R.Nirupama *et al*. 2014). It is used in Ayurvedic and unani systems for the treatment of tumoursand tuberculosis glands. Several Steroidal lactones based on Withanolide have been isolated from the leaves of *Withania somnifera* (Sarangi, A.*et al*.2013). Medicinal plants are a laboratory of biosynthesis not only for chemical compounds but also a mass of the compound from the early times, herbs have been used for healing purposes and most people in the world still use herbs as medicines for different diseases. A lot of plants synthesize substances that are helpful for maintaining health in humans and other animals. Numerous herbs used by humans to season food produce beneficial medicinal compounds.

Herbalism is a traditional practice of medicinal or popular medicines based on the use of herbs and herbal extracts. The WHO has estimated that over 80% of the world's population, in some aspects of primary healthcare, uses medicinal herbs. Approximately 35,000 to 70,000 plant species are estimated to be used as medicinal plants from among 422,127 plant species (Qaiser jamal *et al*. 2013). Plants with one or more organs containing substances that may be used for therapeutic purposes are called herbal remedies (Anubha Arora 2013). The growing failure of chemotherapies and antibiotic resistance of microbial pathogens has led to the detection of potential antimicrobial activity in medicinal plants (Santhi *et al*.2011). Plants and herbal medicines remains a source of proven medicines and revolutionary drugs. They contain substances known to modern old civilizations for their curative properties. Herbal medicine is an important part of all traditional health practices around the world (Sumathi, S. *et al*. 2006). This is an important herbal medicines used for centuries in Ayurvedic medicines to increase longevity and vitality. The versatile use of *Withania somnifera* is caused by Secondary metabolites found in the leaves of the plants (Ara *et al.2012).* The Sanskrit name for this most commercially valuable species is Ashwagandha. The Ashwagandha refers to the smell of horses. The crushed leaves look like the urinary smell of the horse and are consumption of Ashwagandha is also thought to give power as a horse. On the other hand, the Latin word for the species '*Somnifera'* means 'including sleep' and are represents its sedative attribute. Application of this Ashwagandha can be found in *Ashwalayana grahya Sutra and Shatapats Brahman* as ancient text belong to the Vedic period in India (Nimali achini *et al.*2020).Two types of *Asgand* have been referred to in the classical unani literature are *Asgand Nagori* is privileged because of its more possible medicinal properties (uddin *et al*.2012). The estimated production of *Withania somnifera* roots in India is 1,500 tones and the annual requirements are about 7,000 tonnes requiring more cultivation and higher production (Umadevi *et al.* 2012). Aswagandhais a small woody shrub. In Ayurveda, it is an important herbbased Rasayana and is known by the name of 'Sattvic Kapha Rasayana' (Dar *et al*. 2015). It is used for a very long time for all ages groups and both sexes and even while pregnant without any side effects (gupta *et al*. 2007). It is used in over 100 formulations in Ayurveda, Unani, Siddha (krutika J *et al*. 2016). The leaves are used for medicinal purpose. Steroid lactones, called withanolide production in the plant could be monitored by seasonal changes or growing season (kalra*et al*. 2017). In the present study, we evaluated antibacterial activity of leaves of Withania somnifera using a methanolic extracts.

2. MATERIALS AND METHODS

2.1 Sample Collection of *Withania somnifera*

The plants of *Withania somnifera* were collected from the town of Muradnagar, Ghaziabad. The sampling was carried out during the month of March and April.

2.2 Test microorganisms

The following two bacterial pathogenic strains were taken: *Bacillus subtilis* and *Escherichia coli.* These bacterial strains were obtained from stock culture of Institute of Applied Medicines and Research, Duhai, Ghaziabad.

2.3 Extraction and isolation of compounds from leaves of *Withaina somnifera*

Fresh leaves of *Withania somnifera* were collected and washed under running tap water, shade dried and used for extraction these leaves of plants were kept in room for drying for 15to 25 days at room temperature. The dried leaves were crushed by electric glinder. The powdered form of plant material was stored in air tight bottles protected from sunlight until required for analysis.Then 80g crushed leaves were soaked in 200ml methanol. These solvents were used for the extraction of active compounds. The specimens were filtered through muslin cloth and soluble extract were collected in 500ml flask. The solvent were evaporated in the room temperature for 7 days. So finally 7g of crude extracts of leaves was obtained in small bottles. Then crude extracts were stored in refrigerator for further use.

2.4 Loading of Extracts in vials

We can use a four sterilized vials and each vials contains DMSO (Dimethyl sulfoxide) and add an extract in each vials in grams. In one vial contain 0.1g, second vial contains 0.2g, third vial contains 0.4g and fourth vial contains 0.8g extract of *Withania somnifera* and then added a Whattman filter paper no. 4 disc in each vials for 24hrs at room temperature.

2.5 Preparation of petri plates

Suspended 7g of nutrient agar powder in 250ml distilled water and heat the mixture while stirring to fully dissolve all components and then Autoclave the dissolved mixture at 121ºC for 15min.The nutrient Agar has been autoclaved, allow it to cool but not solidify. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified and label a name of plants and culture and concentration in each petri plates. Dip a swab in Bacillus subtilis and Escherichia coli culture and spreading over the each plate and then applied a disc of each concentration in each petri plates. The petri plates were then incubated at 37ºC in the incubator for 24hrs. After 24hrs of incubation, the calliper was used to measure the zone of inhibition for each plant extract and the result was recorded.

3. RESULTS AND DISCUSSION

Inorganic fraction of *Withania somnifera* showed antibacterial activity, while organic fraction had no antibacterial activity. *Bacillus subtilis* was inhibited by methanol extract with an inhibition zone of 17mm while Escherichia coli was inhibited by the methanol extract with inhibition zone of 15 mm (Table 1). Mahesh and Satish (2008) used the methanol extracts of leaf of *Tinospora cordifolia, Ziziphus mauritiana, Withania somnifera, Acacia nilotica and Sida cordifolia* that showed considerable bioactivity against *Staphylococcus aureus, Xanthomonas axonopodis pv. Malvacearum, Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli*. The leaf extract of these plants also showed antifungal activity against *Fusarium verticillioides, Dreschlera turcica* and *Aspergillus flavus.Bacillus subtilis* was highly inhibited by leaf extract of *A. nilotica* and *S. cordifolea*. All the tested bacteria were highly inhibited by leaf extract of *S.cordifolia*. kumar and vinoth examine(2011) examine the leaf samples of Withania somnifera for their antimicrobial potential against some human pathogenic bacteria (*E.coli*, *Bacillus* and *Shigella*) growth inhibition was observed in different volumetric concentration of this extract. These result confirm the antimicrobial property of Withaina somnifera leaf support the traditional use of the plant in therapeutic use against microbial infections.

Table: Antibacterial activity of *Withania somnifera* leaves along with positive control (Gentamicin and Ofloxacin)

Sample	Solvent	Zone of inhibition (mm)									
		Bacillus subtilis					Escherichia coli				
Withania somnifera Leaves	Methanol	100 mg/ml	200 mg/ml	400 mg/ml	800 mg/ml	Positive control (Ofloxacin)	100mg/ml	200 mg/ml	400mg/ml	800 mg/ml	Positive control (Gentamicin)
		8 mm	12mm	15mm	17mm	37mm	7 mm	11mm	13 mm	15mm	29mm

Figure's 2: Zone of inhibition against Bacillus subtilis and Escherichia coli. Circles indicate the inhibition zones by plant extracts

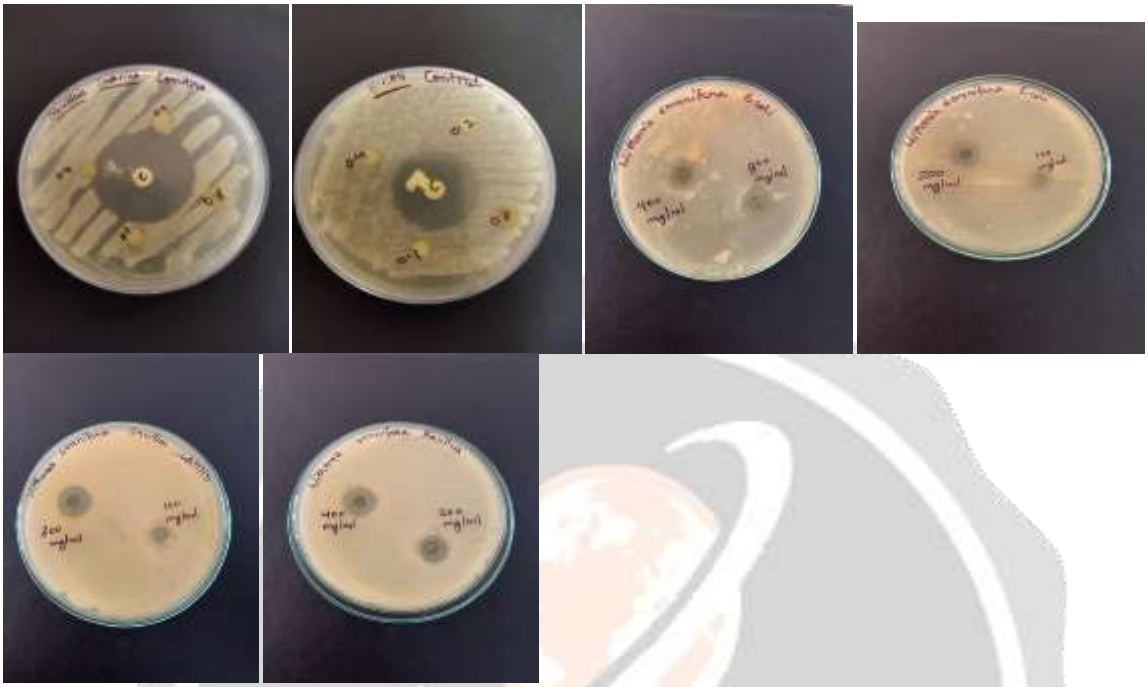
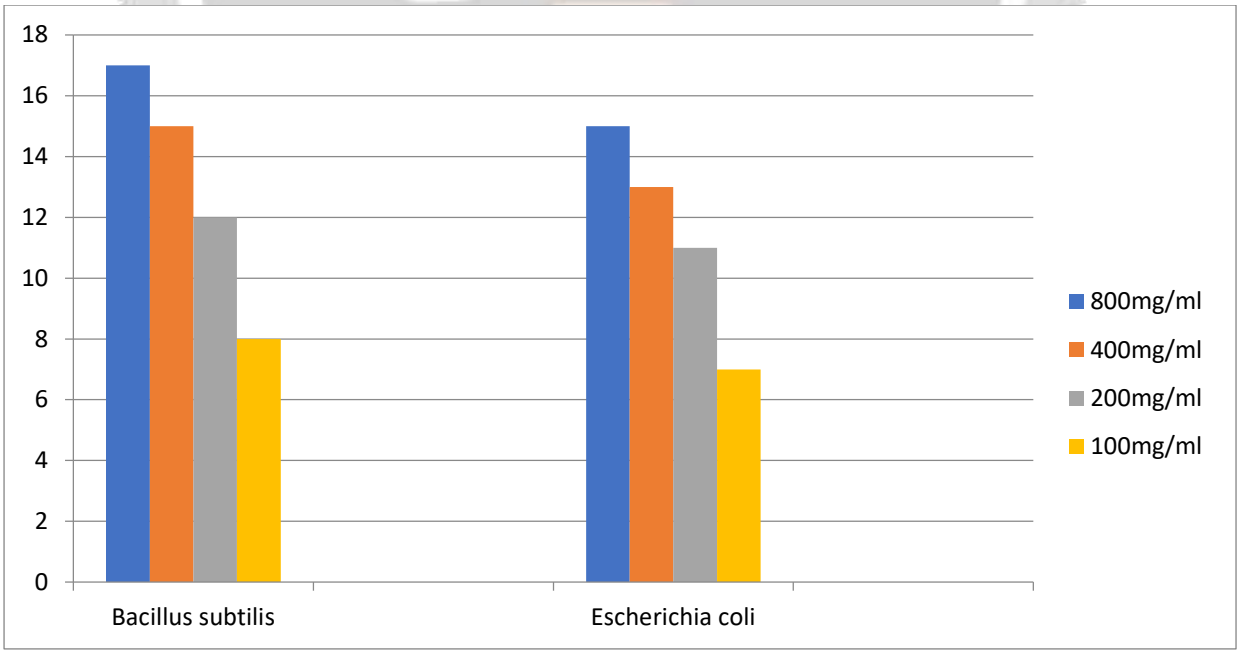


Figure 3: Antibacterial activities against *Withania somnifera*



4. CONCLUSION

Plant extract have great potential as antibacterial compound against bacteria. It is concluded from the present study that the methanolic extract of *Withania somnifera* might be exploited as natural drugs for the treatment of many infectious diseases caused by these organisms.

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