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Research Article

EVOLUTION OF ANTIMICROBIAL ACTIVITY OF *WITHANIA SOMNIFERA* AQUEOUS EXTRACT AGAINST HUMAN PATHOGENS

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ABSTRACT
Withania somnifera (L.) is popularly known as 'Aswagandha' has been an important herb in India and some Mediterranean countries. The fresh leaves were subjected to in vitro antibacterial activity against *Streptococcus pyogenes* (ATCC® 19615™), *Streptococcus pneumoniae* (ATCC 49619), *S. saprophyticus* (ATCC®15305), *Hafnia alvei* (ATCC 51873), *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), *Proteus mirabilis* (ATCC 35659), *Serratia marcescens* (ATCC 274), *Staphylococcus aureus* (ATCC® 25923). *Withania somnifera* (L.) aqueous extract demonstrated highest MIC and MBC effect against *Streptococcus pneumoniae.* Aswagandha aqueous extract showed less bactericidal activity against *S. saprophyticus* and *Enterococcus faecalis pathogens.* Our findings suggest that an appropriate bioactive compound may be developed from *Withania somnifera* (L.) as alternate to antibiotics.

Keywords: *Withania Somnifera, Antibacterial Activity, Solanaceae, Bioactive Compound*

INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The WHO estimated that more than 80% population of the world for some aspect of primary health care use herbal medicines (Jamal *et al.*, 2013).
Withania somnifera Dunal belongs to the family solanaceae. It is a xerophytic plant, found in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions. These shrub common names were Winter cherry in English and Kaknaj-e-Hindi in Persian (Qamar *et al.*, 2012). It is distributed in Sistan & Blochestan province.
Withania somnifera is one of the major herbal components of geriatric tonics mentioned in Indian systems of medicine (Wagner *et al.*, 1994). The roots of *Withania somnifera* consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (Panax ginseng) known as ginsenosides (Verma and Therapeutic, 2011). Phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, phenolic compounds and flavonoids in extracts (Santhi and Swaminathan, 2011). Also chemical constituents of *Withania somnifera* are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, ithasomniferols A-C, withanone etc (Abraham *et al.*, 1968).
It is used as a highly esteemed rasayana drug which is capable of imparting long life, youthful vigor and good intellectual powers; cures ulcers, fever, cough, dyspnoea, consumption dropsy, impotence, rheumatism, toxicosis, leucoderma (Adaikkappan *et al.*, 2012) hiccup, dropsy, gynaecological disordersan as a sedative in senile debility. It is also useful in inflammatory, conditions and scabies as external application. Leaves used as a febrifuge and applied to lesions painful swellings, and sore eyes (Mahesh and Satish, 2008). *Withania somnifera* used for its antioxidant, memory-improving and analgesic effects. It shows relaxant and antispasmodic effects against several plasmogens on intenstinal, uterine, blood vascular, bronchial and tracheal muscles. It used for Tumours, sexual weakness, scrofula, rheumatism, anxiety neurosis, generalized weakness, spermatorhhoea (Imtiyaz *et al.*, 2013). The leaves of *Withania somnifera* are good source of anti-microbial components and roots too are effective in cyto-toxic activities

(Viji, 2011). Withanolides possess remarkable antibacterial, antiarthritic and immunosuppressive. The anti-tumor and radio sensitizing effects of *W. somnifera* have been studied (Singariya *et al.*, 2012).

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Withania somnifera* (Linn.) Dunal (winter cherry) were collected from Medicinal Plant Collection, Institute of Agriculture, University of Zabol, Zabol, Iran, at November 2014.

Figure 1: Plant of *Withania somnifera* in Medicinal Plant Collection

Preparation of Plant Aqueous Extracts

The plant material was washed under running tap water; shade dried in room temperature and powdered using mechanical grinder (Singaria *et al.*, 2012). For aqueous extraction, 10 g of plant powder was dissolved in 100 ml of distilled water in a conical flask, boiled at 100^{o}C in a water bath for 6 hours and then filtered through WhatmanNo.1 filter paper. Prior to use the prepared samples were preserved at 4-5°C in an airtight bottle in refrigerator.

Bacterial Strains

A collection of nine test organisms of American Type of Culture Collection (ATCC), including *Streptococcus pyogenes* (ATCC® 19615™), *Streptococcus pneumoniae* (ATCC 49619), *S. saprophyticus* (ATCC®15305), *Hafnia alvei* (ATCC 51873), *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), *Proteus mirabilis* (ATCC 35659), *Serratia marcescens* (ATCC 274), *Staphylococcus aureus* (ATCC® 25923) were tested for antibacterial study.

Broth Dilution Method for Evaluation of Antibacterial Activity

Minimum inhibitory concentration (MIC) was determined for each organ plant extract showing antimicrobial activity against test pathogens. To measure the MIC values, various concentrations of the stock, 500, 250, 125, 62.5 and 31.25 ppm were assayed against the test pathogens.1ml of each extract was added to test tubes containing 1 ml of sterile NA media (for bacteria). The tubes were then inoculated with standard size of microbial suspension (for bacteria 1×108 CFU/ml) and the tubes were incubated at 37°C for 24 h for bacteria in a BOD incubator and observed for change in turbidity after 24 h compared with the growth and in controls. A tube containing Nutrient broth and inoculum but no extract was taken as control. The least extract concentration which inhibited the growth of the test organisms was taken as MIC. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. Each extract was assayed in duplicate and each time two sets of tubes were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the test tubes. The MIC values were taken as the lowest concentration of the extracts in the test tubes that showed no turbidity after incubation. The turbidity of the test tube was interpreted as visible growth of microorganisms (Singariya *et al.*, 2012).

Equal volume of the various concentration of each extract and nutrient broth mixed in micro-tubes to make up 0.5 ml of solution. 0.5ml of McFarland standard of the organism suspension was added to each tube. The tubes were incubated aerobically at 37^{o}C for 24 h for bacteria. Two control tubes were maintained for each test batch. These include tube containing extract without inoculum and the tube

containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration. MBC was calculated for some of the extracts showed high antimicrobial activity against highly sensitive organisms (Singariya *et al.*, 2012).

RESULTS AND DISCUSSION

Results

Table 1 shows the results of antibacterial activity of the extracts against bacteria. The presence of bioactive compounds in plants has been reported to confer resistance against microbial pathogens and therefore explains the demonstration of antibacterial activity by the plant extracts (Nabeel *et al.*, 2013).

Table 1: Antibacterial activity of the *W. somnifera* aqueous extract

Bacteri	MIC (ppm)	MBC (ppm)	Antibiotic resistance
S.aureus	125	250	E, CE, TE
S.pyogenes	250	250	-
S.pneumoniae	62.5	125	E, CE, CF
H.alvei	250	250	E, TE
S.saprophyticus	250	500	E, CF, TE
A.baumannii	250	250	CE, TE
E.faecalis	250	500	E, CE
P.mirabilis	125	250	E, TE
S.marcescens	125	250	CE

MIC= Minimum Inhibitory Concentration: i.e., the lowest concentration of antibacterial agent that reduces the viability of the initial bacteria inoculums by 99/9%. MBC= Minimum Bactericidal concentration; i.e., the lowest concentration of a particular antibiotic needed to kill bacteria. E= Erythromycin, CE= Cefixime, CF= Ceftazidime, TE= Tetracyclin.

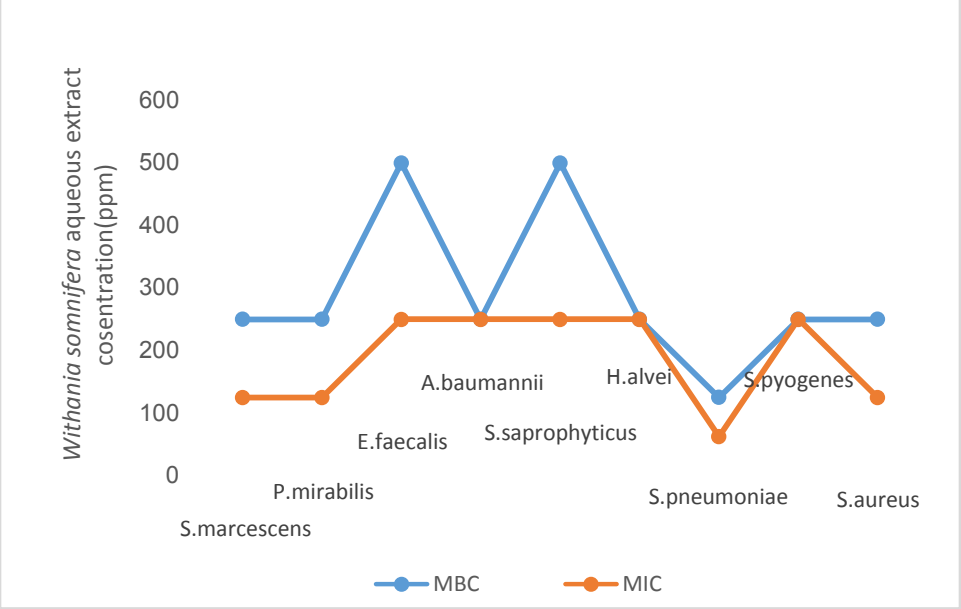

Figure 2: Antibacterial activity of the *W. somnifera* against different pathogens

The results of the present study revealed that *Withania somnifera* (L.) aqueous extract demonstrated highest MIC effect against *Streptococcus pneumonia* (62/5 ppm) followed by *S. aureus*, *P. mirabilis*, *S. marcescens* (125 ppm) and lowest MIC effect against other patogens(500 ppm). Aswagandha aqueous

extract showed highest bactericidal activity against *Streptococcus pneumonia* (125 ppm) and less bactericidal activity against *S. saprophyticus* and *Enterococcus faecalis* pathogens.

Discussion

The results of the present study revealed that *Withania somnifera* (L.) aqueous extract demonstrated highest MIC effect against *Streptococcus pneumonia*(62/5 ppm) followed *by S. aureus, P. mirabilis, S. marcescens* (125 ppm) and lowest MIC effect against other patogens(500 ppm). Aswagandha aqueous extract showed highest bactericidal activity against *Streptococcus pneumonia* (125 ppm) and less bactericidal activity against *S. saprophyticus* and *Enterococcus faecalis* pathogens. Mahesh and Satish (2008) demonstrate that Root and leaf extract of *Withania somifera* showed almost similar antibacterial activity against all the tested bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Xanthomonas axonopodis* pv. Malvacearum) (Mahesh and Satish, 2008). Singariya *et al.*, (2012) study showed that, the range of MIC and MBC of *W. somnifera* extracts recorded was 0.489-15 mg/ml. In this investigation lowest MIC value 0.489 mg/ml was recorded for glacial acetic acid extract against P. *merabilis* and *K. pneumoniae* and followed by 0.938 mg/ml for ethanol, ethyl acetate, chloroform and toluene extract against *A. tumefaciens* indicating significant antimicrobial potential of test extracts (Singariya *et al.*, 2012). Bokaeian *et al.*, (2014) studied the inhibitory effects of leaf extract from *W. somnifera* against *S. aureus*. The highest minimum inhibitory concentrations (MIC) values of extract were found to be 250 ppm against 12 strains and the least value was 62 ppm against 2 strains (Bokaeian and Saeidi, 2015).

MIC of the extract of natural root of the *Withania somnifera* inhibited and fully prevented the growth of *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimurium* and *Klebsicela pneumonia* at of 5.0 mg/ml, *E. coli* and *Staphylococcus aureus* at a concentration of 1mg/ml, *Proteus vulgaris* at a concentration of 40mg/ml (Adhikari *et al.*, 2013). Most of the extracts of *W. somnifera* showed high values of total antibacterial activity (TA) against *P. aeruginosa* and *B. subtilis*. In *W. somnifera* maximum TA values were calculated in water solvent, for unripen fruit extracts (57.96ml) followed by calyx extracts (55.58 ml) and ripen fruit (54.33 ml) against *P. aeruginosa* (Singaria *et al.*, 2011). In other research the stem extract exhibited more inhibition zone than those of leaf and root extract (Sinha, 2012). The inorganic extract of *W. somnifera* leaves showed more antibacterial activity as compared to the organic fraction. While both the organic and inorganic phases of fruit extract of *W. somnifera* showed antibacterial activity against all the tested microorganisms. *S.epidermitis* and *B.subtillis* were inhibited by inorganic fraction of fruit extract. Gentamicin showed lesser activity as compared to inorganic fraction of fruit extract of *W.somnifera* against all the tested microorganisms (Jamal *et al.*, 2013). Plant extracts from *W. somnifera* had inhibitory effect against *K. pneumoniae*. The MIC values were also determined against all the tested bacteria. The highest MIC values of extract were found to be 250 ppm against *K. pneumoniae* and two of MIC value for *K. pneumoniae* was 63 ppm (Bokaeian *et al.*, 2014). *Withania somnifera* plant extract showed more inhibitory activity on gram positive organisms (*Staphylococcus aureus* and *Bacillus cereus*) when compared to gram negative microorganisms (Srinu *et al.*, 2012) this results had agreement with our study. In conclusion, bioactive compounds from *Withania somnifera* extracts could be used as an alternate to antibiotics, considering the side effects and escalating levels of antibiotic resistance among microorganisms.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

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