Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

ANTIBACTERIAL POTENTIAL OF ALKALOIDS OF *WITHANIA SOMNIFERA* L. & *EUPHORBIA HIRTA* L.

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Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

ABSTRACT

Alkaloid extracts of different parts (root, stem, leaf & fruits) of *Withania somnifera* & *Euphorbia hirta* showed significant antibacterial activity against *Enterobacter aerogens*, *Bacillus subtilis*, *Klebsiella pneumoniae, Raoultella planticola* and *Agrobacterium tumefaciens* . Results from the disc diffusion assay showed that stem alkaloid extract of *W. somnifera* showed highest antibacterial activity (IZ 25.5 mm; AI 1.159±0.023) against *Enterobacter aerogens.* Alkaloids of root of *W. somnifera* recorded significant activity against all the test bacteria. The minimum inhibitory concentration (MIC) of *W. somnifera* was 0.039 mg/ml against *E. aerogens*, *K. pneumoniae* & *A. tumefaciens,* where as, in the case of *E. hirta* lowest MIC 0.039 mg/ml was observed against *E. aerogens*, *R. planticola* & *A. tumefaciens.* Higher total activity 474.35 ml/g was observed for *R. planticola* & *A. tumefaciens* in *E. hirta.* The results reveal that studied plant is potentially a good source of antibacterial agents & support the traditional applications of the tested plants (*W. somnifera* & *E. hirta*).

Keywords: *W. somnifera, E. hirta*, Antibacterial activity, IZ, MIC, Total activity.

INTRODUCTION

Although pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs in microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen. 1992). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multidrug-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, (Montelli and Levy. 1991) documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around the world. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem and one of the ways to overcome this problem is to encourage research to develop new drugs, which might be synthetic or natural. Since the synthetic drugs are mostly associated with side effects, hence more emphasis should be given to develop safe, natural plant based drugs. According to World Health Organization (Santos et al 1995) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Eloff 1998). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of plants. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this study was to evaluate the potential of plant extracts and phytochemicals on standard microorganism strains as well as multi-drug resistant bacteria, which were isolated from hospitals. Further the synergistic effects of extracts for antimicrobial activity in association with antibiotics against drugs resistant bacteria. Two plants, namely, *Withania somnifera* (Solanaceae) and *Euphorbia hirta* (Euphorbiaceae) were selected in the present study, for evaluation of their antibacterial activities.

Review of literature reveals that no work has been carried out extraction and screening of specific compound from selected plants is concerned as far as. Hence, in the present work an extraction and screening for antibacterial activity of the alkaloids of *W. somnifera* &*E. hirta* has been undertaken.

MATERIALS & METHODS

Preliminary detection of alkaloids

Each of the test samples was acidified by 5 ml of 2% HCl at 60°C for 2 h and later cooled and filtered. Formation of the white precipitate on the addition of the following reagents, individually to 2ml of the above solution indicated the presence of alkaloids.

Extraction of Alkaloids

Alkaloids were extracted from different parts of the selected plants by well established methods (Harborne. 1984) after preliminary detection of alkaloids. Finely powered sample (100g) of plant parts were extracted with 10% acetic acid in ethanol for 4 h. Extracts were concentrated and were made alkaline by NH_4OH. Precipitate thus obtained was collected by centrifugation, washed with 1% NH_4OH, filtered, dried in vaccuo and weighed. Extracts thus obtained were stored at 4°C in air tight glass vials for further use.

Selected Test Microorganisms

Pathogenic microorganisms selected for study include five bacteria, viz., *E. aerogens* (MTCC 2822), *B. subtilis* (MTCC 121), *K.* pneumoniae (MTCC 4030), *R. planticola* (MTCC 2271) and *A. tumefaciens* (MTCC 431). Selected microorganisms were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on "Muller- Hinton Agar Medium".

Antimicrobial screening of extracts

Disc diffusion assay (DDA) was performed for antimicrobial screening. (Gould et al., 1952; Andrews, 2001). MH agar (for bacteria) and SD agar (for fungi) base plates were seeded with the standard inoculums size of bacteria (1×10^8 CFU/ml). Sterile filter paper discs (6mm in diameter) were impregnated with 100µl of each of the extract (10mg/ml concentration) to give a final concentration of 1mg/disc, left to dry in vaccuo to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates.

Each extract was tested in triplicate along with streptomycin (1mg/disc). The plates were kept at 4°C for 1h for diffusion of extract, thereafter were incubated at 37±2°C for 24 h. Zone of inhibition (IZ) or depressed growth of microorganisms was measured and the 'Activity Index' (AI) for each extract was calculated.

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal / Fungicidal Concentration (MBC/MFC)

Minimum inhibitory concentration (MIC) was determined for plant extract showing antimicrobial activity against test pathogens in Disc Diffusion Assay. Broth microdilution method (Barsi et al., 2005) was followed for determination of MIC values. Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two fold serial dilution. Thereafter 100µl inoculum of standard size was added to each well. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 37±2°C for 24h. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms. The minimum bacterial/fungicidal concentration (MBC/MFC) was determined by subculturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC.

Total activity (TA)

Total activity is the volume at which test extract can be diluted without loosing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g (Eloff, 2004). In mathematical terms it can be expressed as:

$$\text{Total activity (TA)} = \frac{\text{Amount extracted from 1g plant material}}{\text{MIC of the extract}}$$

RESULTS & DISCUSSION

The data pertaining to the antimicrobial potential of the plant extracts are tabulated in Table 1, 2, 3 & shown in Fig. 1, 2, 3, respectively.

Table 1: Inhibition zone and Activity index of extracts of *W. somnifera* L. & *E. hirta* L.

Microorganisms		***B. subtilis***		***R. planticola***		***E. aerogens***		***K.* pneumoniae**		***A. tumefaciens***	
Plants	**Plant parts**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**
W. somnifera	Leaf	-	-	8.75	0.291± 0.025	9.25	0.420± 0.011	15.75	0.926± 0.015	15.75	0.562± 0.009
	Stem	8.25	0.458± 0.014	-	-	25.5	1.159± 0.023	13.75	0.809± 0.015	13.25	0.473± 0.027
	Root	8.75	0.486± 0.014	10.75	0.358± 0.025	11.75	0.534± 0.012	14.25	0.838± 0.015	15.25	0.545± 0.045
	Fruits	-	-	9.75	0.325± 0.009	-	-	-	-	11.75	0.419± 0.027
E. hirta	Leaf	-	-	12.75	0.425± 0.025	12.25	0.557± 0.012	12.25	0.720± 0.015	13.75	0.491± 0.027
	Stem	-	-	10.25	0.342± 0.008	10.25	0.466±0.012	15.75	0.926± 0.015	13	0.464± 0.036
	Root	-	-	8.75	0.292± 0.009	9	0.409± 0.023	12.75	0.750± 0.015	10.75	0.384± 0.009
	Fruits	-	-	-	-	-	-	11.75	0.691± 0.015	12.25	0.437± 0.009

IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc),
AI= Activity Index (IZ developed by extract/ IZ developed by standard),
± = SEM,
(-) = No activity
Extracts assayed in triplicate
IZ of standard drug streptomycin against),
E. aerogens(22mm), *B. subtilis* (18mm), *K. pneumoniae (17mm), R. planticola* (30mm), *A. tumefaciens (28mm).*

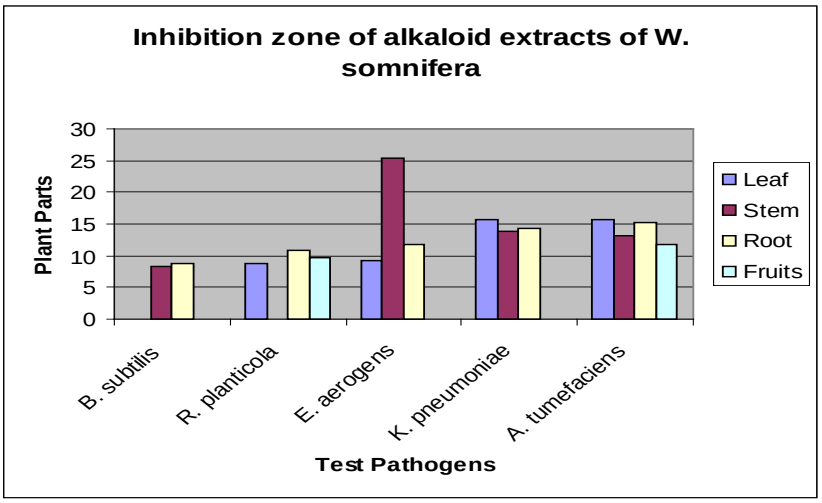

Fig. 1: Inhibition zone of alkaloids extracts of *W. somnifera*

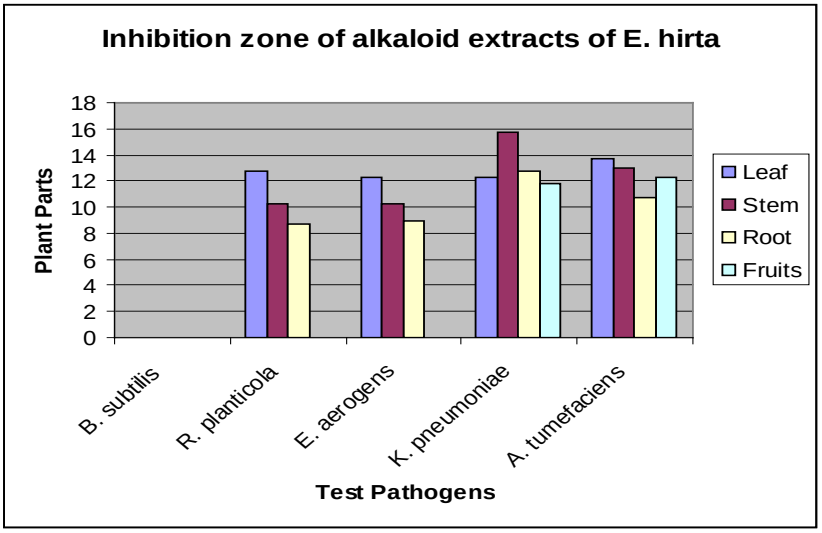

Fig. 2: Inhibition zone of alkaloids extracts of *E. hirta*

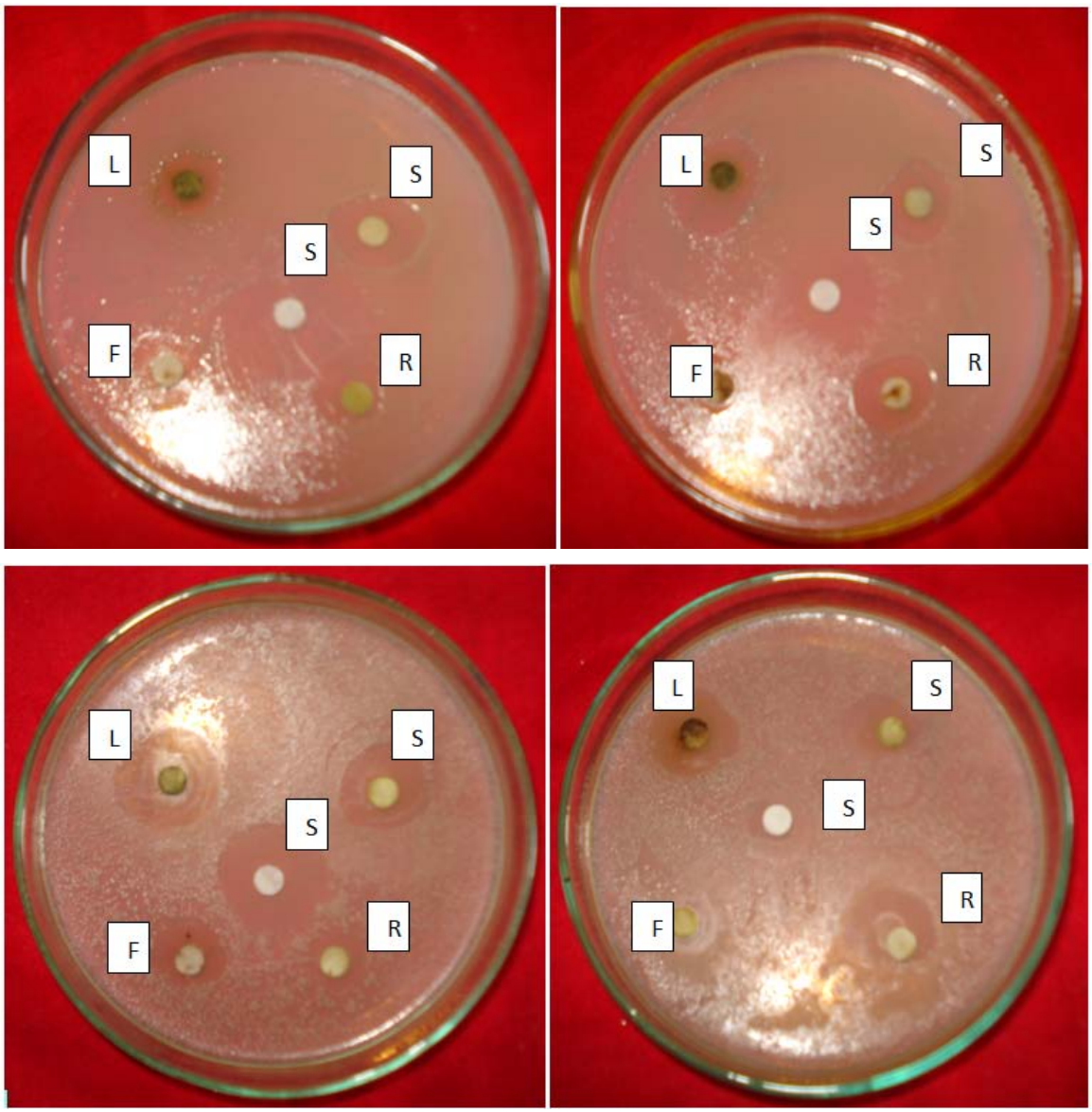

Fig. 3: Inhibition Zone of extracts of selected plants against microorganisms

S: Standard Disc; L = Leaf; S= Stem; R= Root; F= Fruit
1. *Euphobia hirta* / *Klebsiella pneumoniae*
2 Withania somnifera / *Klebsiella pneumoniae*
3. *Euphobia* hirta / *Agrobacterium tumefaciens*
4. *Withania somnifera* / *Agrobacterium tumefaciens*

In the present investigation total 8 extracts were tested, & all of them showed antibacterial activity. Among all most susceptible organisms in the investigation was *A. tumefaciens* against which all the plant extracts showed inhibition zone. All selected five pathogens were inhibited by alkaloids of roots of *W. somnifera*. Most of the extracts showed bioactivity against more than two microorganisms tested. The minimum inhibition zone (IZ 25.5 mm; AI 1.159±0.023; MIC 0.039mg/ml) was observed by stem alkaloids of *W. somnifera* against *E. aerogens*, where as stem alkaloids of *E. hirta* showed significant activity (IZ 15.75mm; AI 0.926±0.015; MIC 0.078mg/ml) against *K.* pneumoniae. Leaf alkaloid of *W. somnifera* showed same activity (IZ 15.75mm) against *K.* pneumoniae & *A. tumefaciens* (Table 1).Most resistant microorganism observed under present investigation was *B. subtilis* against which only two extracts showed bioactivity. The range of MIC & MBC of extracts recorded was 0.039-0.625mg/ml & 0.039-1.25mg/ml, respectively. In the case of *W. somnifera* bactericidal effect was found against *E. aerogens* & *B. subtilis* whereas in the case of E. hirta bactericidal effect was observed for *A. tumefaciens* & *K.* pneumoniae.

Table 2: MIC and MBC of extracts of *W. somnifera* L. & *E. hirta* L.

Microorganisms		***B. subtilis***		***R. planticola***		***E. aerogens***		***K.* pneumoniae**		***A. tumefaciens***	
Plants	**Plant parts**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
W. somnifera	Leaf	-	-	0.625	1.25	0.312	0.625	0.039	0.156	0.039	0.156
	Stem	0.312	0.312	-	-	0.039	0.039	0.039	0.078	0.078	0.156
	Root	0.625	1.25	0.078	0.312	0.039	0.078	0.039	0.156	0.039	0.078
	Fruits	-	-	0.156	0.312	-	-	-	-	0.156	0.312
E. hirta	Leaf	-	-	0.039	0.078	0.078	0.156	0.312	0.625	0.039	0.039
	Stem	-	-	0.156	0.625	0.156	0.312	0.078	0.078	0.078	0.156
	Root	-	-	0.625	1.25	0.312	0.625	0.156	0.312	0.312	1.25
	Fruits	-	-	-	-	-	-	0.312	0.625	0.078	0.156

MIC = Minimum Inhibitory Concentration (mg/ml)

MBC = Minimum Bactericidal (mg/ml)

Total activity (TA) as a measure of of potency was also determined. Most potent extract under study was leaf alkaloid of *E. hirta*, which showed high value of total activity (474.35 ml/g) against *R. planticola* & *A. tumefaciens.*

Include references of work done by others on these plants. Since the selected plants have shown good potency, hence can be exploited in future for preparation of an alternative drug for multi drug resistant bacteria at commercial scale.

Table 3: Total activity of the extracts of *W. somnifera* & *E. hirta*

Plants	**Plant parts**	**Quantity of extract mg/g dried plant part**	**Total Activity (ml/g)**				
			B. subtilis	***R. planticola***	***E. aerogens***	***K.* pneumoniae**	***A. tumefaciens***
W. somnifera	Leaf	7	-	11.2	22.43	179.48	179.48
	Stem	10.5	33.65	-	269.23	269.23	134.61
	Root	12	19.2	153.84	307.69	307.69	307.69
	Fruits	43	-	275.64	-	-	275.64
E. hirta	Leaf	18.5	-	474.35	273.17	59.29	474.35
	Stem	7.5	-	48.07	48.07	96.15	96.15
	Root	28.5	-	45.6	91.34	182.69	91.34
	Fruits	13.5	-	-	-	43.26	173.07

Total activity= Extract per gram dried plant part; MIC

ACKNOWLEDGEMENT

Authors are thankful to the Head of Botany Department, University of Rajasthan for providing all necessary facilities for present work. Financial assistance provided by UGC is gratefully acknowledged.

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