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Research Article

COMPARATIVE MICROCIDAL ACTIVITY OF *WITHANIA SOMNIFERA* AND *CENCHRUS SETIGERUS* AGAINST THE PATHOGENIC MICRO-ORGANISMS

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ABSTRACT

Crude extracts of different parts of *Withania somnifera* (RUBL-20668) and *Cenchrus setigerus* (CAZRI-76) were successively extracted with polar to non polar solvents using soxhlet assembly. The extracts were then screened for their Biological activity *in-vitro* against one Gram positive bacteria (*Staphylococcus aureus*), two gram negative bacteria (*Escherichia coli* and *Raoultella planticola*) and one yeast (*Candida albicans*) by disc diffusion assay. Serial dilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). Water extract of leaves of *W. somnifera* showed highest activity against *R. planticola* and chloroform extract of stem of *C. setigerus* showed highest activity against *S. aureus.*

Keywords: *Cenchr*us *setigerus, Withania somnifera, Raoultella planticola, Escherichia coli, Candida albicans* and Antimicrobial.

INTRODUCTION

Herbal remedies represent one of the most important fields of traditional medicines especially in tribal areas and phyto-therapy is practiced by large group of tribal for treatment of several physical, physiological and social ailments[1]. Recently due to increase in antibiotic resistant strains of clinically important pathogens, a line of new bacterial strains that are multi drug resistant[2-4] have emerged. Further non-availability and high cost of new generation antibiotics have resulted in increase in morbidity and mortality rate[5]. Therefore, there is an urgent need to search for new compounds and/or sources with proven antimicrobial activity.

W. somnifera (Family Solanaceae) is one of the commonest plant species used for the treatment of candidiasis[6]. It is used for the treatment of arthritis, tuberculosis, cancer and sexual transmitted infections[7-9]. The antimicrobial properties of this plant species have been widely reported in literatures[10-11].

Cenchrus L. (Poaceae) is highly nutritious grass and considered excellent for pasture in hot, dry areas and is valued for its production of palatable forage and intermittent grazing during droughty periods in the tropics. *C. setigerus* is more efficient at gathering Carbon dioxide, utilizing nitrogen from the atmosphere and recycled N in the soil[12-13]. This C_4 grass is more competitive under the conditions of high temperature, solar radiation and low moisture[14]. The grass, fed green, turned into silage, or made into hay is said to increase flow of milk in cattle and impart a sleek and glossy appearance. This grass has excellent soil binding capacity which helps to conserve soil in desert areas[15]. Although, *C. setigerus* is most suitable and highly nutritive grass for desertic conditions, still no antimicrobial work has yet been reported.

E. coli, *S. aureus* and *C. albicans* have been proved to be the major causal organisms for various human infections and have been selected for the present study. *E. coli* and *S. aureus* causes a variety of suppurative, wound infections and food poisoning in human beings. Major causative agent of nosocomial infections is *S. aureus*[16]. *E. coli* and *R. planticola* have been reported to cause severe [17]. Present investigation has been carried out to evaluate antibacterial and anticandidal effects of crude extracts of *C. setigerus* and *W. somnifera*. The study was carried out along with the standard drugs Gentamycin (for bacteria), Ketoconazole (for yeast).

MATERIAL AND METHODS

Experimental design

Crude extracts of different parts of *W. somnifera* (RUBL-20668) and *C. setigerus* (CAZRI-76) were separately extracted with a series of non polar to polar solvents by hot extraction method[18] in soxhlet assembly. Different extracts were then screened for antimicrobial activity by 'Disc diffusion Assay'[19] against a few medically important bacteria and yeast. The fraction showing best activity was then used for determining 'Minimum inhibitory concentration' (MIC) by serial dilution method[20] and 'Minimum bactericidal/fungicidal concentration' (MBC/MFC).

Collection of plant material

Different parts of *C. setigerus* (CAZRI-76) were collected in the month of August from the Central Arid Zone Research Institute, Jodhpur, Rajasthan and parts of *W. somnifera* (RUBL-20668) were collected in the month of January from Jaipur district of Rajasthan. Plants samples were identified and deposited in the herbarium, department of botany, university of Rajasthan, Jaipur. The collected plant materials were transferred immediately to the laboratory cleaned with water and selected plant parts were separately shade dried for one week. Each shade dried plant part was powdered with the help of grinder. Fine powder of each sample was stored in clean container to be used for Soxhlet extraction following the method of Subramanian and Nagarjan, (1969)[21] in different polar solvents selected.

Extraction procedure

Each plant part (10 gm) was sequentially extracted with different solvents (250 ml) according to their increasing polarity (Benzene < Chloroform < Water) by using Soxhlet apparatus for 18 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No. 1 filter paper. The extracts solutions were evaporated under reduced pressure at 40 °C[22]. The residual extracts were stored in refrigerator at 4^0C in small and sterile glass bottles. Percent extractive values were calculated by the following formula (table-1).

$$\text{Percent Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Drugs and chemicals used

Drugs

Gentamycin (for bacteria) and Ketoconazole (for yeast)

Chemicals

Benzene, Chloroform, Water, Nutrient Agar (for bacteria) Sabouraud Dextrose Agar (for yeast).

Micro-organisms

Bacteria

Escherichia coli (MTCC-46),

Staphylococcus aureus (MTCC-3160),

Raoultella planticola (MTCC-530).

Yeast

Candida albicans (MTCC 183).

Screening for antimicrobial activity

Test pathogenic microorganisms were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on Nutrient Agar medium, while yeast was maintained on Sabouraud Dextrose Agar medium. Disc diffusion assay[19] was performed for screening. Sterile filter paper discs (Whatman no. 1, 5mm in diameter) were impregnated with 100 µl of each of the extract (10 mg/ml) to give a final concentration of 1 mg/disc and left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination. A bacterial suspension was prepared and inoculum size 1×10^8 CFU/ml was added for bacteria and 1×10^7 cell/ml for yeast[23] to the sterilized medium before solidification. The media with bacteria was poured into sterilized Petri dishes under aseptic condition[24]. Extract discs were then placed on the seeded agar plates. Each extract was tested in triplicate with gentamycin (10mcg/disc) and ketoconazole (10mcg/disc) as standard for bacteria and yeast, respectively. The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 37°C for bacteria (24 h) and at 27°C for yeast (48 h)[6]. After incubation the average of inhibition zones was recorded[25-26]. Inhibition zones were measured and average size was compared with IZ of standard reference antibiotics and Activity index for each extract was calculated by following formula and recorded (Table 2).

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

Determination of minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration (MIC) was evaluated as the lowest concentration with no visible growth of test pathogens[6]. To measure MIC, various concentrations of the stock, 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117, 0.059, 0.029 mg/ml were assayed against the test pathogens. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 15mg/ml final concentration and then two fold serial dilution (1 ml of each extract was added to test tubes containing 1 ml of sterile Nutrient Agar media for bacteria and Sabouraud Dextrose Agar media for yeast. The tubes were then inoculated with standard size of microbial suspension (for bacteria 1×10^8 CFU/ml and 1×10^7 cell/ml for yeast) and the tubes were incubated at 37°C for 24 h for bacteria and 27°C for 48 h for yeast in a BOD incubator and were observed for change in turbidity and compared with the growth in controls[27]. A tube containing nutrient broth and inoculum but no extract was taken as control. Bacterial and fungal 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.

Determination of Minimum bactericidal/fungicidal concentration (MBC/MFC)

Equal volume of various concentration of each extract and nutrient agar were mixed in micro-tubes to make up 0.5ml of solution. Then 0.5ml of McFarland standard of the organism suspension was added to each tube[28]. The tubes were incubated aerobically at 37°C for 24 h for bacteria and 27°C for 48 h for yeast. 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 Nutrient Agar followed by incubation. The highest dilution that yielded no single pathogen was taken as the Minimum bactericidal Concentration[29]. MBC was calculated for those extracts that had shown high antimicrobial activity against tested organisms.

Total activity (TA) determination

Total activity is the volume at which the test extract can be diluted with 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[30].

$$\text{Total Activity} = \frac{\text{Extract per gram dried plant part}}{\text{MIC of extract}}$$

Statistical Analysis

Mean value and standard deviation were calculated for each test bacteria and yeast. Data were analyzed by one-way ANOVA and p values were considered significant at $p > 0.005$[31].

RESULTS

Preliminary phyto–profiling

The preliminary phyto–profiling for the different parts of *W. somnifera* (RUBL-20668) and *C. setigerus* (CAZRI-76) were carried out according to Farnsworth (1966)[32], wherein the consistency was found to be non-sticky in the high polar solvent extracts whereas the low polar solvent extracts were found to be sticky. The highest yield (w/w mg/10gm) was recorded for leaf extracts of *W. somnifera* in water (2495.65±18.67 mg/10gm) Table no. 1.

Antimicrobial activity

Antimicrobial activity (denoted in terms of inhibition zone and activity index) of plant extracts, tested against selected microorganisms were recorded (Table 2). In the present study total 24 extracts of different parts of selected plants were tested for their bioactivity. Seventeen extracts showed significant antimicrobial potential against test microbes. However 7 extracts showed no activity against any of the selected microorganisms at the tested concentration (four from *C. setigerus* and three from *W. somnifera*). Most susceptible organism in the investigation was *C. albicans* against which, most of the plant extracts showed inhibition zone. Maximum Antimicrobial activities were recorded in leaf extract of different polar solvents of *W. somnifera.*

Antibacterial activity

Leaf extracts of *W. somnifera* showed maximum antibacterial activity against pathogen in all polar solvents. Maximum activity was observed in water solvent (IZ-21.83±0.25 mm, AI-1.092) against *R. planticola* followed by benzene (IZ-19.83±0.22 mm, AI-0.992) and chloroform solvent IZ-15.83±0.26 mm, AI-0.792) against *S. aureus.* Benzene and water extracts of *C. setigerus* did not show any antibacterial activity (Table 2).

Antifungal activity

Maximum antifungal activity was observed for water extracts of both the selected plants, leaf extracts of *W. somnifera* (IZ of 12.17±0.26 mm, AI 1.739) followed by root extracts of *C. setigerus* (IZ of 10.5±0.64 mm, AI 0.656) against *C. albicans*. All the benzene and chloroform extracts of *C. setigerus* showed no bioactivity against *C. albicans* (Table 2).

MIC and MBC/MFC

MIC and MBC/MFC values (Table 3) were recorded for those plant extracts, which had shown activity in disc diffusion assay. Range of MIC and MBC/MFC recorded was 0.938 - 15 mg/ml. In the present investigation lowest MIC values were recorded for *W. somnifera* leaf extract in water (0.938 mg/ml) against *R. planticola* and *C. albicans* followed by chloroform and benzene (1.875 mg/ml) against *S. aureus*. MIC of chloroform extract of *C. setigerus* leaf, root and stem also observed to be same (1.875 mg/ml) against *S. aureus* indicating significant antimicrobial potential of test extracts.

Table 1: Preliminary phyto-profile and Total activity of different parts of *Cenchrus setigerus* and *Withania somnifera*

Solvents	Parts	Yield mg/10gm±S.D.	Color	Consistency	Total activity of test pathogens			
					E.c.	*S.a.*	*R.p.*	*C.a.*
***Cenchrus setigerus* (CAZRI-76)**								
	R	87±8.86	Yellow	Nonsticky	-	-	-	-
Benzene	S	193±13.46	Yellow	Nonsticky	-	-	-	-
	L	179±13.37	Dark brown	Sticky	-	-	-	-
	Se	121±15.63	Yellow	Sticky	-	-	-	-
	R	322±13.42	Brown	Sticky	8.59	17.17	8.59	-
Chloroform	S	357±9.64	Greenish brown	Sticky	9.52	19.04	9.52	-
	L	419±11.85	Green	Sticky	-	11.17	5.59	-
	Se	389±8.95	Yellow	Sticky	2.59	20.75	-	-
	R	215±12.94	Dark brown	Nonsticky	-	-	-	5.73
Water	S	119±9.37	Brick red	Nonsticky	-	-	-	3.17
	L	197±11.75	Dark coffee	Sticky	-	-	-	5.25
	Se	202±11.37	Dark brown	Nonsticky	-	-	-	2.69
***Withania somnifera* (RUBL-20668)**								
	R	156.15±6.89	Parrot green	Sticky	-	4.16	-	-
Benzene	S	67.75±5.77	Greenish brown	Sticky	-	3.61	0.9	7.22
	L	580.20±8.23	Parrot green	Sticky	-	30.94	-	-
	C	368.90±7.45	Green	Sticky	-	-	-	-
	R	666.90±13.76	Dark brown	Nonsticky	-	-	-	-
Chloroform	S	496.75±9.47	Dark green	Nonsticky	-	13.25	-	26.49
	L	1176.05±14.69	Dark green	Sticky	-	62.72	-	62.72
	C	573.10±8.97	Dark green	Sticky	-	-	-	-
	R	928.60±12.53	Pale green	Nonsticky	-	-	24.76	49.53
Water	S	1473.45±14.36	Light green	Nonsticky	-	-	19.65	78.58
	L	2495.65±18.67	Brown	Nonsticky	-	-	266.06	266.06
	C	2195.10±16.89	Green	Nonsticky	-	-	-	117.07

R- Root; S- Stem; L- Leaf; Se- Seed; C- Calyx

E. c. - Escherichia coli; S. a. - Staphylococcus aureus

R. p. - Raoultella planticola; C. a. - Candida albicans

Table 2: Inhibition zone (mm) and Activity index for different parts of *C. setigerus* and *W. somnifera* against tested pathogens.

Solvents	Polarity of Solvents	Plant Part	Test microorganisms *Cenchrus setigerus* (CAZRI-76)							
			Escherichia coli		*Staphylococcus aureus*		*Raoultella planticola*		*Candida albicans*	
			IZ±S.D.	AI	IZ±S.D.	AI	IZ±S.D.	AI	IZ±S.D.	AI
		Root	-	-	-	-	-	-	-	-
Benzene	2.7	Stem	-	-	-	-	-	-	-	-
		Leaf	-	-	-	-	-	-	-	-
		Seed	-	-	-	-	-	-	-	-
		Root	8.33±0.24	0.417	11.17±0.22	0.698	8.67±0.24	0.434	-	-
Chloroform	4.1	Stem	9.17±0.27	0.459	14.5±0.64	0.906	9.5±0.64	0.475	-	-
		Leaf	-	-	8.67±0.24	0.542	8.17±0.23	0.409	-	-
		Seed	7.33±0.23	0.367	12.5±0.64	0.781	-	-	-	-
		Root	-	-	-	-	-	-	10.5±0.64	0.656
Water	9	Stem	-	-	-	-	-	-	9.33±0.23	0.583
		Leaf	-	-	-	-	-	-	9.67±0.25	0.604
		Seed	-	-	-	-	-	-	8.17±0.24	0.511
***Withania somnifera* (RUBL-20668)**										
		Root	-	-	9.50±0.64	0.475	-	-	-	-
Benzene	2.7	Stem	-	-	13.17±0.23	0.659	7.17±0.23	0.398	11.17±0.25	1.596
		Leaf	-	-	19.83±0.22	0.992	-	-	-	-
		Calyx	-	-	-	-	-	-	-	-
		Root	-	-	-	-	-	-	-	-
Chloroform	4.1	Stem	-	-	8.33±0.27	0.417	-	-	8.67±0.27	1.239
		Leaf	-	-	15.83±0.26	0.792	-	-	11.83±0.22	1.69
		Calyx	-	-	-	-	-	-	-	-
		Root	-	-	-	-	17.50±0.65	0.875	9.33±0.23	1.333
Water	9	Stem	-	-	-	-	10.67±0.24	0.534	9.50±0.64	1.357
		Leaf	-	-	-	-	21.83±0.25	1.092	12.17±0.26	1.739
		Calyx	-	-	-	-	-	-	8.67±0.29	1.239

*All values are mean ± SD, n=3 (p>0.005).

Table 3: MIC and MBC/MFC of different parts of *C. setigerus* and *W. somnifera* against tested pathogens

Solvents	Plant Part	Test microorganisms *Cenchrus setigerus* (CAZRI-76)							
		E. coli		*S. aureus*		*R. planticola*		*C. albicans*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
	R	-	-	-	-	-	-	-	-
Benzene	S	-	-	-	-	-	-	-	-
	L	-	-	-	-	-	-	-	-
	Se	-	-	-	-	-	-	-	-
	R	3.75	7.5	1.875	3.75	3.75	7.5	-	-
Chloroform	S	3.75	3.75	1.875	1.875	3.75	3.75	-	-
	L	-	-	3.75	7.5	7.5	15	-	-
	Se	15	15	1.875	3.75	-	-	-	-
	R	-	-	-	-	-	-	3.75	3.75
Water	S	-	-	-	-	-	-	3.75	7.5
	L	-	-	-	-	-	-	3.75	3.75
	Se	-	-	-	-	-	-	7.5	15
***Withania somnifera* (RUBL 20668)**									
	R	-	-	3.75	7.5	-	-	-	-
Benzene	S	-	-	1.875	3.75	7.5	15	0.938	1.875
	L	-	-	1.875	1.875	-	-	-	-
	C	-	-	-	-	-	-	-	-
	R	-	-	-	-	-	-	-	-
Chloroform	S	-	-	3.75	7.5	-	-	1.875	3.75
	L	-	-	1.875	1.875	-	-	1.875	1.875
	C	-	-	-	-	-	-	-	-
	R	-	-	-	-	3.75	3.75	1.875	1.875
Water	S	-	-	-	-	7.5	7.5	1.875	1.875
	L	-	-	-	-	0.938	0.938	0.938	0.938
	C	-	-	-	-	-	-	1.875	1.875

R- Root; S- Stem; L- Leaf; Se- Seed; C- Calyx

MIC - Minimum inhibitory concentration (mg/ml)

MBC - Minimum bactericidal concentration (mg/ml)

MFC - Minimum fungicidal concentration (mg/ml)

Total activity

Total activity indicates the volume at which extract can be diluted with still retaining ability to kill microorganism (Table 1). Most of the extracts of *W. somnifera* and *C. setigerus* showed high values of TA against *C. albicans* and *S. aureus* respectively, which proves the potential of extracts to inhibit growth of the test microorganisms, even at low concentration. In *W. somnifera* maximum TA values were calculated for leaf extract in water (266.06 ml) against *R. planticola* and *C. albicans* followed by *C. setigerus* for seed (20.75 ml) and stem extracts (9.52 ml) in chloroform against *S. aureus* and *E. coli* respectively.

DISCUSSION

Results of the present study revealed that 17/24 plant extracts tested, inhibited the growth of selected bacteria and fungi, indicating broad spectrum bioactive nature of selected two plants (9/12 in *W. somnifera* and 8/12 in *C. setigerus*). It indicates that *W. somnifera* is more potential than *C. setigerus* as far as bioactivity concerned. In general, leaf extracts of *W. somnifera* and stem extracts of *C. setigerus* express maximum antibacterial and antifungal activities by suppressing the growth of all microbes under investigation. Excellent antibacterial and antifungal activities were observed for water extracts of *W. somnifera* and chloroform extracts of *C. setigerus*, due to low MIC and MBC/MFC values. MBC/MFC values were found higher than the MIC values of the extracts against microorganisms tested; indicate the bacteriostatic/fungistatic effects of the extracts. Chloroform extracts of stem in *C. setigerus* were recorded as bactericidal against *R. planticola, S. aureus* and *E. coli*. On the other hand, all the extracts of *W. somnifera* in water expressed fungicidal nature against *C. albicans*. Gram positive bacteria *S. aureus* was the second most susceptible organism after *C. albicans*, which supported the finding that plant extracts are usually more active against Gram positive bacteria than Gram negative[33-34]. Susceptibility differences between Gram-positive and Gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including synthetic and natural antibiotics[35].

Extracts under study not only inhibit the bacterial/fungal growth but the IZ developed, was more or less permanent when compared with the IZ developed by the standard drug used, as after sometime bacterial/fungal colonies could be easily seen in IZ developed by standard drugs. In the light of the fact that microorganism are becoming resistant against the drugs in use, present investigation is of great significance, as far as the future plant based drugs are concerned and choice of selected plants by the pharmaceutical industries for preparing plant based antimicrobials drugs.

C. setigerus easily grow in harsh climate or xeric conditions and requires less care; hence its use as raw material for preparing drugs would definitely be economical, when commercial production of plant based drug in concerned.

CONCLUSION

In the present study total 24 extracts of different parts of desert grasses and *W. somnifera* were tested for their bioactivity, among which 17 extracts showed significant antimicrobial potential against test microbes. Compared to reference antibiotics, the spectrum of antibacterial activity of investigated plants was found superior. The demonstration of broad spectrum of *W. somnifera* and *C. setigerus* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. The effect of these plants on more pathogenic organisms, toxicological investigations and further purification, however, need to be carried out.

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REFERENCES

1. Swarnkar S, Katewa SS: Antimicromrobial Activities of some Tuberous Medicinal Plants from Aravalli Hills of Rajasthan. J of Herbal Medicine and Toxicology 2009; 3(1): 53-58.
2. Thambiraj J, Paulsamy S: Antimicrobial Screening of Stem Extract of the Folklore Medicinal Plant, *Acalypha fruticosa* Forssk. Int J Pharm Pharm Sci 2011; 3(4): 285-287.
3. Aibinu IE, Ohaegbulam VC, Adenipekun EA, Ogunsola FT, Odugbemi TO, Mee BJ: Extended-Spectrum Beta-Lactamase Enzymes in Clinical Isolates of *Enterobacter* species from Lagos, Nigeria. Journal of Clinical Microbiology 2003; 41(5):2197-2200.
4. Aibinu I, Adenipekun E, Odugbemi T: Emergence of Quinolone Resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. Nigerian J of Health and Biomedical Science 2004; 3(2):73-78.
5. Williams R: Antimicrobial resistance a global threat. Essential Drug Monitor 2000; 1: 28-29.
6. Kambizi L, Afolayan AJ: Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea.* African J of Biotech 2008; 7(1): 12-15.
7. Devi PU: *Withania somnifera* Dunal (Ashwagandha): Potential plant source of a promising drug for cancer chemotherapy and radiosensitization. Indian J Exp Biol 1996; 34: 927-932.
8. Van Wyk BE, Van Oudtshoorn B, Gercke N: Medicinal Plants of South Africa. Briza, Pretoria 1997; 1-304.
9. Singh S, Kumar S: *Withania somnifera*: The Indian ginseng ashwagandha. Central Institute of Medicinal and Aromatic Plants 1998; 293.
10. Afolayan AJ, Grierson DS, Kambizi L, Madamombe I, Masika PJ: *In vitro* antifungal activity of some South African medicinal plants. S Afri J Bot 2002; 68: 72-76.
11. Arora S, Dhillon S, Rani G, Nagpal A: The *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. Fitoterapia 2004; 75: 385-388.
12. Bessman SP: Ammonia Metabolism in Animals: Symposium on Inorganic Nitrogen Metabolism. Mc Elry and Glass (eds.) 1956; The Johns Hopkins Press.
13. Singariya P: Effect of Sub-Optimal Environment and PGR's on Metabolic Pattern of Certain Species of *Cenchrus*. Ph.D Thesis, 2009; J. N. Vyas University.
14. Agrawal P: Ecophysiological and Biochemical studies Related to drought adaptation in grasses of Indian Desert. Ph.D. Thesis, 2007; J. N. Vyas University.
15. Sinha RK, Bhatia S, Vishnoi R: Desertification control and rangeland management in the Thar desert of India. In: Rala Report no. 200, 1996; 115-123.
16. Alvarez LF, Paloman M, Insausti J, Olaechea P, Cerda F, Sanchez GJ, De La Torre MV : *Staphylococcus aureus* nosocomial infections in critically ill patients admitted in intensive care units. Med Clin (Barc) 2006; 126(17): 641-646.
17. Alves MS, Riley LW, Moreira BM: "A case of severe pancreatitis complicated by *Raoultella planticola* infection". J Med Microbiol 2007; 56: 696–698.
18. Harborne JB: Phytochemical Methods, Chapman andHall, 2nd edn. London, 1984, New York 5-6.
19. Andrews JM: BSAC standardized disc susceptibility testing method. J Antimicrob Chemother 2001; 4: 43-57.
20. Joan Stokes E: Clinical Bacteriology, Edward Arnold, 1975; London.
21. Subramanian SS, Nagarjan S: Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. Current Sci 1969; 38: 65.
22. Bokhari FM: Antifungal activity of some medicinal plants used in Jeddah Saudi Arabia. Mycopath 2009; 7(1): 51-57
23. Baker CN, Thornsberg CH: Inoculum standardization in antimicrobial susceptibility tests: Evaluation of overnight age culture. J Chin Microbiol 1983; 17: 140-457.
24. Hussain AA, Mohammed AA, Ibrahim HH, Abbas AH: Study the Biological Activities of *Tribulus Terrestris* Extracts. World Academy of Science, Engineering and Technology 2009; 57: 433-435.
25. Kandil O, Radwan NM, Hassan AB, Amer AM, El- Banna HA: Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. J Ethnopharmacol 1994; 44(1): 19-24.
26. McCan D, Kirkis L: Evaluation of Free Testosterone in serum. J Clin Immunoassay 1985; 8: 234-236.
27. Hanumantharaju N, Shashidhara S, Rajashekharan PE, Rajendra CE: Comparative Evaluation of Antimicrobial and Antioxidant Activities of *Kaempferia galangal* for Natural and Micropropagated Plant. Int J Pharm Pharm Sci 2010; 2(4): 72-75.
28. Shahidi Bonjar GH: Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus, Serratia marcescens*, *Klebsiella pneunomiae* and *Bordella bronchoseptica*. Asian J of Sci 2004; 3(1): 82-86.
29. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA: Screening of crude extracts of six medicinal plants used in southwest Nigerian unorthodox medicine for antimethicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine 2005; 5:6.
30. Eloff JN: Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. Phytomedicine 2004; 11(4): 370-371.
31. Jain T, Sharma K: Assay of antibacterial activity of *Polyalthia longifolia* Benth. And Hook. Leaf extracts. J of Cell and Tissue Res 2009; 9(2): 1817-1820.
32. Farnsworth NR: Biology and Phytochemical screening of plants. Pharm Sci 1966; 55: 225-276.
33. Lin J, Opake AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jäger AK: Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethanopharmacol 1999; 68: 267-274.
34. Palombo EA, Semple SJ: Antibacterial activity of traditional Australian medicinal plants. J Ethnopharmacol 2001; 77: 151-157.
35. Tortora GJ, Funke BR, Case CL: Microbiology: An Introduction, Benjamin Cummings (2001); San Francisco.