Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

EVOLUTION OF INDIAN GINSENG AGAINST DIFFERENT BACTERIA AND FUNGI

PREMLATA SINGARIYA*[1], PADMA KUMAR[1] AND KRISHAN K. MOURYA[2]

[1] Dr. D.S. Kothari Post doctoral fellow, Laboratory of Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur- 302004, [2]Veterinary Officer & In-charge, I[st] Gr. Govt. Veterinary Hospital, Pahari (Bharatpur) Rajasthan
Email: premlatasingariya@gmail.com

Received: 2 February 2012, Revised and Accepted:6 May 2012

ABSTRACT

Different parts (root, stem, leaf, flower, unripen fruit, ripen fruit and calyx) of *Withania somnifera* (RUBL-20668) were evaluated for their antimicrobial (antibacterial and antifungal) properties. The dried and powdered parts were successively extracted using soxhlet assembly then antibacterial and antifungal activities were investigated with by both disc diffusion and serial dilution methods against the *Pseudomonas aeruginosa* (Gram-ve), *Bacillus subtilis* (Gram+ve), *Enterobactor aerogens* (Gram-ve) and one fungi *Aspergillus flavus*. The extract of *W. somnifera* significantly inhibited these important bacteria and fungi to varying degrees. Maximum extracts were showed highest activity against *P. aeruginosa* and *B. subtilis*. The inhibitory effect is very identical in magnitude and comparable with that of standard antibiotics. Gentamycin, the standard antibacterial drug used was effective in inhibiting these bacteria. The effect on *P. Aeruginosa*, *B. subtilis* and *E. aerogens* were comparable to that of gentamycin. Ketoconazole, the standard antifungal used was effective against the fungi (*A. flavus*).

Keywords: *Withania somnifera*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobactor aerogens* and *Aspergillus flavus*.

INTRODUCTION

Natural plants derived compounds contribute a lot in fight against pathogens[1]. Various plant extracts have also been examined for their antibacterial activity with the objective of exploring environmentally safe alternatives of plant disease control[2]. Antimicrobial resistance to anti microbial agents has lead to treatment failure and the shift of medical care from orthodox to herbal medicine.

W. somnifera used in significant increase hemoglobin concentration, red blood cell count, white blood cell count, platelet count and body weight as compared to controls, as well as increased hemolytic antibody responses towards human erythrocytes[3], Anti-inflammatory effect, analgesic effect, osteoarthritis[4], immuno-potentiating and myelo-protective effect[5], increased phagocytic activity and prolonged survival time[6], antifungal activity of Withania has been confirmed elsewhere, attributed to the withanolides.

P. aeruginosa is involved in respiratory tract, urinary tract[7], bloodstream, and central nervous system infections of nosocomial origin[8] and this pathogen is becoming resistant against gentamycin, ciprofloxacin[9] tetracycline, chloramphenicol, and norfloxacin[10]. *Bacillus Subtilis* can contaminate food; however, they seldom result in food poisoning. *E. aerogens* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections.

MATERIAL AND METHODS

Experimental design

Crude extracts of different parts of *W. somnifera* (RUBL-20668) were prepared with a series of non polar to polar solvents by hot extraction method[11] in soxhlet assembly. Different extracts were then screened for antimicrobial activity by disc diffusion Assay[12] against a few medically important bacteria, fungi and fungi. The fraction showing best activity was then used for determining of minimum inhibitory concentration (MIC) by tube dilution method[13] and minimum bactericidal/fungicidal concentration (MBC/MFC).

Collection of plant material

Different parts of *W. somnifera* 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 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 cleancontainer to be used for Soxhlet extraction following the method of Subramanian and Nagarjan[14] 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 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 and then concentrated at 40^0C by using an evaporator. The residual extracts were stored in refrigerator at 4^0C in small and sterile glass bottles.

Drugs and chemicals used

Drugs

Gentamycin and Ketoconazole as standard antibiotics for bacteria and fungi respectively.

Chemicals

Petrolium ether, ethyl acetate, glacial acetic acid, Mueller-Hinton Agar, Nutrient Agar (NA) (for bacteria), Sabouraud Dextrose Agar (SDA)(for fungi).

Micro-organisms

Test pathogenic microorganisms were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar slants, sub cultured regularly (after every 30 days) and stored at 4^oC as well as at -80^oC by preparing suspensions in 10% glycerol.

Bacteria

Pseudomonas aeruginosa (Gram-ve) (MTCC-1934), *Bacillus subtilis* (Gram+ve) (MTCC-121), *Enterobactor aerogens* (Gram-ve) (MTCC-111).

Fungi

Aspergillus flavus (MTCC-277)

Screening for antimicrobial activity

Initial screening of different stem extracts for their antibacterial activity carried out using Mueller-Hinton and Nutrient agar media did not reveal any significant difference, thus further studies were carried out using nutrient agar medium only[15]. Bacterial strains were grown and maintained on Nutrient Agar medium, while fungi were maintained on SDA medium. Disc diffusion assay performed for screening. NA and SDA base plates were seeded with the bacterial and fungal inoculum, respectively (inoculum size 1×10^8 CFU/ml for bacteria and 1×10^7 cell/ml for fungi). Sterile filters paper discs (Whatman no. 1, 5mm in diameter) were impregnated with

100 µl of each of the extracts (100 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. The ZOI were measured and compared with the standard reference antibiotics. AI for each extract was calculated.

$$\text{Activity index (AI)} = \frac{\text{Zones of inhibition (ZOI) of the sample}}{\text{Zones of inhibition (ZOI) of the standard}}$$

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against test pathogens. 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 (serial dilution method), 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[16]. The turbidity of the test tube was interpreted as visible growth of microorganisms.

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

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[17]. MBC was calculated for some of the extracts showed high antimicrobial activity against highly sensitive 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[18,19].

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

RESULTS

1. Quantitative estimation

The preliminary phyto–profiling for the different parts of *W. somnifera* were carried out according to Bokhari[20] wherein the consistency was found to be sticky in the high polar solvent extracts whereas the low polar solvent extracts were found to be non-sticky. The yield (mg/gm) of the extracts was also analyzed wherein the highest yield was recorded for Calyx (344.455 mg/gm) followed by unripen fruit (337.285 mg/gm) in glacial acetic acid extract (Table 1).

Table 1: zone of inhibition (mm)* and activity index for different parts of *w. somnifera*

Solvents With Polarity	Plant Part	Test microorganisms							
		Pseudomonas aeruginosa		*Bacillus subtilis*		*Enterobactor aerogens*		*Aspergillus flavus*	
		ZOI±S.D.	AI	ZOI±S.D.	AI	ZOI±S.D.	AI	ZOI±S.D.	AI
Petrolium Ether (0.1)	Root	8.17±0.23	0.817	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-
	Leaf	20.83±0.24	2.083	-	-	-	-	9.67±0.24	0.967
	Flower	-	-	8.17±0.24	0.454	-	-	-	-
	Unripen Fruit	-	-	15.33±0.22	0.852	-	-	8.50±0.64	0.850
	Ripen Fruit	-	-	12.50±0.65	0.694	-	-	-	-
	Fruit coat (Calyx)	-	-	12.67±0.25	0.704	-	-	-	-
Ethyl Acetate (4.4)	Root	9.5±0.64	0.792	10.17±0.24	0.565	-	-	-	-
	Stem	8.67±0.21	0.723	12.33±0.25	0.685	-	-	-	-
	Leaf	13.83±0.24	1.153	20.83±0.28	1.157	-	-	8.83±0.23	0.736
	Flower	7.17±0.22	0.598	-	-	-	-	-	-
	Unripen Fruit	8.33±0.28	0.694	12.50±0.65	0.694	-	-	-	-
	Ripen Fruit	7.50±0.64	0.625	9.33±0.25	0.518	-	-	-	-
	Fruit coat (Calyx)	8.67±0.22	0.723	14.50±0.65	0.806	-	-	-	-
Glacial Acetic Acid (6.2)	Root	7.17±0.24	0.598	25.50±0.64	1.417	8.17±0.21	0.545	8.67±0.26	0.788
	Stem	8.17±0.21	0.681	9.67±0.24	0.537	9.33±0.23	0.622	7.5±0.64	0.682
	Leaf	20.84±0.25	1.737	35.83±0.26	1.991	15.67±0.26	1.045	11.33±0.23	1.030
	Flower	28.67±0.26	2.389	37.67±0.24	2.093	34.83±0.24	2.322	14.17±0.21	1.288
	Unripen Fruit	20.50±0.64	1.708	20.17±0.22	1.121	19.50±0.65	1.3	9.50±0.65	0.864
	Ripen Fruit	18.67±0.24	1.556	14.33±0.23	0.796	16.67±0.23	1.111	10.66±0.22	0.969
	Fruit coat (Calyx)	25.83±0.21	2.153	45.33±0.24	2.518	32.50±0.64	2.167	15.83±0.28	1.439

R- Root; S- Stem; L- Leaf; F- Flower; Un- Unripen fruit; Rp- Ripen fruit and C- Calyx; *P. a.- Pseudomonas aeruginosa*; *B. s.-Bacillus subtilis*; *E. a.- Enterobactor aerogens*; *A. f.- Aspergillus flavus*.

2. Antimicrobial activity

Antimicrobial activity [assessed in terms of zone of inhibition (ZOI) in mm* and activity index] of the different parts of *W. somnifera* extracts in different polar solvents, tested against selected microorganisms were recorded (Table 2). In the present study total 21 extracts of selected plant were tested for their bioactivity, among which 20 extracts showed significant antimicrobial potential against test microbes. However, only one extract (in Petrolium ether solvent) showed no activity against any selected microorganism at tested concentration. Highest antibacterial activity was recorded for calyx extract (ZOI- 45.33±0.24 and AI- 2.518) followed by flower extract (ZOI- 37.67±0.24 and AI- 2.093) against *B. subtilis* in glacial acetic acid solvent as well as highest antifungal activity was recorded for calyx extract (ZOI- 15.83±0.28 and AI- 1.439) followed by flower extract (ZOI- 14.17±0.21 and AI- 1.288) against *A. flavus* in the same solvent. Most susceptible organisms in the investigation were *B. subtilis* and *P. aeruginosa* against which, most of the plant extracts showed inhibition zone. Petrolium ether and ethyl acetate extracts did not produce any type of activity against *E. aerogens.*

Table 2: Zone of inhibition (mm)* and activity index for different parts of *w. somnifera*

Solvents With Polarity	Plant Part	Test microorganisms							
		Pseudomonas aeruginosa		*Bacillus subtilis*		*Enterobactor aerogens*		*Aspergillus flavus*	
		ZOI±S.D.	AI	ZOI±S.D.	AI	ZOI±S.D.	AI	ZOI±S.D.	AI
	Root	8.17±0.23	0.817	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-

Petrolium Ether (0.1)	Leaf	20.83±0.24	2.083	-	-	-	-	9.67±0.24	0.967
	Flower	-	-	8.17±0.24	0.454	-	-	-	-
	Unripen Fruit	-	-	15.33±0.22	0.852	-	-	8.50±0.64	0.850
	Ripen Fruit	-	-	12.50±0.65	0.694	-	-	-	-
	Fruit coat (Calyx)	-	-	12.67±0.25	0.704	-	-	-	-
	Root	9.5±0.64	0.792	10.17±0.24	0.565	-	-	-	-
	Stem	8.67±0.21	0.723	12.33±0.25	0.685	-	-	-	-
Ethyl Acetate (4.4)	Leaf	13.83±0.24	1.153	20.83±0.28	1.157	-	-	8.83±0.23	0.736
	Flower	7.17±0.22	0.598	-	-	-	-	-	-
	Unripen Fruit	8.33±0.28	0.694	12.50±0.65	0.694	-	-	-	-
	Ripen Fruit	7.50±0.64	0.625	9.33±0.25	0.518	-	-	-	-
	Fruit coat (Calyx)	8.67±0.22	0.723	14.50±0.65	0.806	-	-	-	-
	Root	7.17±0.24	0.598	25.50±0.64	1.417	8.17±0.21	0.545	8.67±0.26	0.788
	Stem	8.17±0.21	0.681	9.67±0.24	0.537	9.33±0.23	0.622	7.5±0.64	0.682
Glacial Acetic Acid (6.2)	Leaf	20.84±0.25	1.737	35.83±0.26	1.991	15.67±0.26	1.045	11.33±0.23	1.030
	Flower	28.67±0.26	2.389	37.67±0.24	2.093	34.83±0.24	2.322	14.17±0.21	1.288
	Unripen Fruit	20.50±0.64	1.708	20.17±0.22	1.121	19.50±0.65	1.3	9.50±0.65	0.864
	Ripen Fruit	18.67±0.24	1.556	14.33±0.23	0.796	16.67±0.23	1.111	10.66±0.22	0.969
	Fruit coat (Calyx)	25.83±0.21	2.153	45.33±0.24	2.518	32.50±0.64	2.167	15.83±0.28	1.439

***All values are mean ± SD, n=3**

MIC and MBC/MFC

MIC and MBC/MFC values were evaluated for those plant extracts, which were showing activity in diffusion assay. The range of MIC and MBC/MFC of extracts recorded was 0.234-15 mg/ml. In the present investigation lowest MIC value 0.234 mg/ml was recorded for calyx extract in the glacial acetic acid and leaf extract in ethyl acetate against *B. subtilis* followed by 0.438 mg/ml against *P. aeruginosa* by calyx extract, against *B. subtilis* by flower and root extract, and *E. aerogens* by flower and calyx extracts in glacial acetic acid indicating significant antimicrobial potential of test extracts. MIC and MBC/MFC values were found equal show bactericidal and fungicidal activity (table 3).

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

Solvents (% Water Solubility)	Plant Part	Test microorganisms							
		P. aeruginosa		*B. subtilis*		*E. aerogens*		*A. flavus*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Petrolium Ether (7.5)	R	3.75	7.5	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-
	L	0.938	1.875	-	-	-	-	1.875	3.75
	F	-	-	3.75	7.5	-	-	-	-
	Un	-	-	1.875	1.875	-	-	3.75	7.5
	Rp	-	-	3.75	7.5	-	-	-	-
	C	-	-	1.875	3.75	-	-	-	-
Ethyl Acetate (8.7)	R	1.875	3.75	3.75	3.75	-	-	-	-
	S	1.875	3.75	1.875	1.875	-	-	-	-
	L	1.875	3.75	0.234	0.468	-	-	7.5	15
	F	7.5	15	-	-	-	-	-	-
	Un	3.75	7.5	3.75	7.5	-	-	-	-
	Rp	3.75	7.5	3.75	7.5	-	-	-	-
	C	3.75	7.5	1.875	1.875	-	-	-	-
Glacial Acetic Acid (100)	R	7.5	15	0.468	0.468	1.875	3.75	1.875	1.875
	S	3.75	7.5	3.75	7.5	1.875	3.75	1.875	1.875
	L	1.875	1.875	0.468	0.468	0.938	1.875	1.875	3.75
	F	0.938	0.938	0.468	0.468	0.468	0.468	0.938	0.938
	Un	1.875	1.875	0.938	0.938	0.938	0.938	0.938	0.938
	Rp	1.875	1.875	3.75	3.75	0.938	0.938	0.938	1.875
	C	0.468	0.468	0.234	0.234	0.468	0.468	0.938	1.875

R- Root; S- Stem; L- Leaf; F- Flower; Un- Unripen fruit; Rp- Ripen fruit; 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 having ability to kill microorganism. Fruit and calyx extracts showed high values of TA against *P. aeruginosa*, *B. subtilis* and *E. aerogens* which prove the potential to inhibit the growth of the test microorganisms, even at low concentration. Maximum TA values were recorded, 1472.42 ml against *B. subtilis* and followed by 736.21 ml against *P. aeruginosa* and *E. aerogens* by calyx extract in the glacial acetic acid solvent.

Overall, the test pathogens were more sensitive to the glacial acetic acid extracts than to the ethyl acetate and Petrolium ether extract. This suggests that some of the active compounds in the crude extracts are polar and thus dissolved readily in glacial acetic acid while the ethyl acetate and Petrolium ether extract may have dissolved out non-polar compounds that possess less antimicrobial activity.

DISCUSSION

Results of the present study showed that 20/21 extracts tested inhibited the growth of selected bacteria and fungi, indicating broad spectrum bioactive nature of *W. somnifera*. It indicates that *W. somnifera* has broad spectrum bioactive nature. Glacial acetic acid extracts of *W. somnifera* express maximum antibacterial and antifungal activities by suppressing the growth of all microbes under investigation. In the present study, most of the extracts were found to be potent inhibitor of tested organisms except *E. aerogens*.

Excellent antibacterial and antifungal activities were observed by glacial acetic acid extracts were shown by 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. Gram positive bacteria *B. subtilis* and *P. aeruginosa* were the most susceptible organisms, which supported the finding that plant extracts are usually more active against Gram positive bacteria than Gram negative[21,22,13,16,18,19].

In general, the Gram-negative bacteria have shown less sensitivity to plant extracts possibly as a result of their extra lipo-polysaccharide and protein cell wall that provides a permeability barrier to the antibacterial agent[23]. 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[24]. Extracts under study not only inhibit the bacterial/fungal growth but the ZOI developed, was more or less permanent when compared with the ZOI developed by the standard drug used, as after sometime bacterial/fungal colonies could be easily seen in ZOI 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 drugs are concerned and uses of selected plants by the pharmaceutical industries for preparing plant based antimicrobials drugs.

ACKNOWLEDGEMENT

Authors are expressing their thanks to UGC for providing the funds for the project under Dr. D. S. Kothari, Post doctoral fellowship scheme.

REFERENCES

1. Vyvyan JR. Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron 2002;58: 1631-1646.
2. Gracelin D, Herin Sheeba, Britto A, John De. *Datura metel* linn.- A plant with potential as Antimicrobial agent. I. J. of applied biology and pharmaceutical technology 2011;2(2) :429-433.
3. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwarthan B. Studies on the immunomodulatory effects of Ashwagandha. J. Ethnopharmacol 1996;50(2): 69-76.
4. Kulkarni RR, Patki PS, Jog VP, Gandage SG, Patwardhan B. Treatment of osteoarthritis with a herbo mineral formulation: a double-blind, placebo-controlled, cross-over study. J Ethnopharmacol 1991;33(1-2): 91-95.
5. Davis L, Kuttan G. Effect of Withania somnifera on cytokine production in normal and cyclophosphamide treated mice. ImmunoPharmacology and Immunotoxicology 1999;21(4): 695-703
6. Dhuley JN. Effect of ashwagandha on lipid peroxidation in stress induced Animals. J. Ethano Pharmacology 1998;60(2): 173-178.
7. Jones TC, Hunt RD, King NW. Veterinary Pathology, 6th Edn., Lea and Febiger, Philadelphia, 1997.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. 10th Ed.; Mosby, Inc. Elsevier: St. Louis, Missouri, USA, 2007, 205.
9. Gailiene G, Pavilonis A, Kareiviene V. The Peculiarities of *Pseudomonas aeruginosa* Resistance to Antibiotics and Prevalence of Serogroups. Medicina (Kaunas), 43, 2007, 36-42.
10. Li XZ, Livermore DM, Nikaido H. Role of Efflux Pump(s) in Intrinsic Resistance of *Pseudomonas aeruginosa*: Resistance to Tetracycline, Chloramphenicol, and Norfloxacin. Antimicrob. Agents Chemother. 1994;38 :1732-1741.
11. Harborne JB. Phytochemical Methods, Chapman and Hall, 2ed edn. London, New York 1984;5-6.
12. Bawer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J clin pathol, 1966; 45:493-6.
13. Singariya P, Mourya KK, Kumar P. Preliminary Phyto-profile and Pharmacological Evaluation of some Extracts of *Cenchrus* grass against Selected Pathogens. J. Pharm. Sci. and Res 2011a;3(8): 1387-1393.
14. Subramanian SS, Nagarjan S. Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. Current Sci 1969;38: 65.
15. Arora DS, Kaur GJ. Antibacterial activity of some Indian medicinal plants. J Nat Med. 2007; 61:313–317.
16. Singariya P, Mourya KK, Kumar P. Bio Activity of crude extracts of Leaves of *Cenchrus* Grass in different polar solvents against some pathogenic microbes. I. J. of Pharm. Sci. Review and Res 2011b; 11(1): 124-129.
17. 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.
18. Singariya P, Mourya KK, Kumar P. Comparative Microcidal Activity of *Withania somnifera* and *Cenchrus setigerus* against the Pathogenic Micro-organisms. I. Journal of Pharmacy and Pharmaceutical science 2011c; 3(55): 511-515.
19. Singariya P, Mourya KK, Kumar P. Antimicrobial Activity of the Crude Extracts of *Withania somnifera* and *Cenchrus setigerus In-vitro*. Pharmacognosy journal 2011d;3(27): 60-65.
20. Bokhari FM. Antifungal activity of some medicinal plants used in Jeddah Saudi Arabia. Mycopath 2009;7(1) :51-57.
21. 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.
22. Palombo EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants J Ethnopharmacol 2001;77: 151-157.
23. Adwan K, Abu-Hasan N. Gentamicin resistance in clinical strains of Enterobacteriaceae associated with reduced gentamicin uptake. Folia Microbiol 1998;43 :438-40.
24. Tortora G J, Funke BR, Case CL. Microbiology: An Introduction, Benjamin Cummings. San Francisco 2001.