

Antimicrobial Activity of *Girardinia heterophylla*

P. S. Bedi, Neayti Thakur, Balvinder Singh

Abstract—In the present study an attempt has been made to prepare the crude extracts of leaves and stem of '*Girardinia heterophylla*' by using various solvents like petroleum ether, ethanol and double distilled water. The samples were given the code NGLS 1, NGLS 2, NGLS 3 and NGSS 1, NGSS 2 and NGSS 3 respectively. All the extracts were used to study their antimicrobial activity against gram positive bacteria e.g. *Bacillus subtilis*, gram negative bacteria e.g. *E. coli* and *K. pneumonia* and antifungal activity against *Aspergillus niger*. The results of the antimicrobial activity showed that all the crude extracts of the plant possesses antibacterial activity. Maximum antibacterial activity was shown by NGLS 2, NGLS 3 and NGSS 3 against *K. pneumonia*. The growth of fungus *A. niger* was also inhibited by all the crude extracts. Maximum inhibition was shown by NGSS 2 followed by NGSS 1.

Keywords—*Girardinia heterophylla*, leaves and stem extracts, antibacterial activity, antifungal activity.

I. INTRODUCTION

HERBS had been prized for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed importance, for a while. There is evidence of herbs having been used in the treatment of diseases and for revitalising body system in almost all ancient civilizations, the Egyptian, the Chinese and even Greek and Roman civilizations [1], [2]. Nature has been a source of medicinal agents for thousands of years. Medicinal plants have been used to promote good health and treat diseases effecting people. Medicinal plants constitute an effective source of traditional and modern medicines [3]. According to the estimates of WHO, over 80% of people in developing countries depend upon traditional medicine for their primary health care. One possible reason for this is the perception of them having lesser side effects [4], [5]. Traditional treatments may provide valuable clues for the development of new oral hypo-glycemic agents and simple dietary adjuncts, as one of the most important plant *Girardinia heterophylla*, Dence. = *G. diversifolia* [6]. Hindi names: Awa, alla, bichua (means scorpion); English name 'Himalayan Nettle' or 'Stinging Nettle' belongs to family '*Urticaceae*' [7]. The plant has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles that inject histamine and other chemicals that produce a stinging sensation when contacted by humans or animals [8]. The literature is silent or very limited information is available about *Girardinia*

P. S. Bedi is with the Department of Applied Sciences, Chandigarh Group of Colleges, Mohali, Punjab, India (Corresponding author phone: 00919781925248, 00911723984215; e-mail: dean.academics@gmail.com; drbedips@gmail.com).

Balvinder Singh is with the Department of Biochemistry, SBSPGI, Dehradun, India.

heterophylla. Keeping this in view the present study has been under taken.

II. MATERIAL AND METHODS

The sample of leaves and stem of *Girardinia heterophylla* were collected from Solan, Himachal Pradesh, India and given the codes NGL and NGS respectively (Table I).

TABLE I
DETAILS OF CODES USED FOR VARIOUS SOLVENT EXTRACTS OF DIFFERENT PARTS OF THE PLANT

PLANT PART	CODES	SOLVENT USED
<i>Girardinia heterophylla</i> Leaves	NGLS1	Petroleum ether
	NGLS2	Ethanol
	NGLS3	Double distilled water
<i>Girardinia heterophylla</i> Stem	NGSS1	Petroleum ether
	NGSS2	Ethanol
	NGSS3	Double distilled water

A. Determination of Anti Microbial Activity

Many herbs are known to possess antimicrobial properties. The plant "*Girardinia*" is used to treat many diseases. Many patients have been benefited by this herb. Hence the antimicrobial property of the medicine was investigated.

III. ASSAY TECHNIQUES

Various microbiological techniques are available to assay the antimicrobial activity of any compound e.g. Disc diffusion method, Cup plate method, Overlay assay technique etc.

Agar diffusion method (well diffusion method) or (cylinder plate method) was used for antibacterial activity as reported by Kavanagh [9] and Leven [10]. Wells were made in seeded agar and the test sample was then introduced directly into these wells. After incubation, the diameter of the clear zones around each well was measured and compared against zone of inhibition of the known concentrations of the standard antibiotics. The fresh plant materials collected were rinsed with water and kept under shade at room temperature for drying. After complete dryness, these plant parts were ground into powder and were kept in sealed plastic bags duly labeled. Extraction of these plant parts were carried out one by one by simple maceration process.

IV. SAMPLE PREPARATION

Petroleum ether, Ethanol and double distilled water were used to extract bioactive products from leaf and stem of *Girardinia heterophylla*. The test bacterial isolated studied were *Bacillus subtilis* (MTCC-121), *Escherichia coli* (MTCC-1052) and *Klebsiella pneumonia* (MTCC-109), the fungus

Aspergillus niger. These cultures were maintained at culture collection centre of SBSPGI Dheradun.

A. Test Culture Preparation

Test culture were inoculated in nutrient broth (0.5gm NaCl; 0.5gm peptone; 0.3gm beef extract; 1000ml water pH 7) and kept in incubator at 37°C for 24 hours. The fungal strains were inoculated in fungal medium (0.4g Yeast extract; 1.5g Sucrose; 0.2g NaNO₃; 0.001g FeSO₄ · 7 H₂O; 0.05g K₂SO₄; 0.05g MgSO₄; 0.05g KCl; distilled water 100ml 2.5; pH 5.0-5.5) kept in another incubator at 23°C for 48 hours.

V. CUP PLATE METHOD

Sample extracts were poured into the wells dug in the Petri plates of solid agar medium and before fitting the wells with sample supernants the plates were swabbed with test organism cultures on the respective plates i.e. nutrient agar (Nutrient broth with 1.5% agar) for bacterial test cultures and fungal agar medium (fungal medium with 1.5% agar) for fungal culture (*Aspergillus niger*).

VI. RESULTS AND DISCUSSION

A. Antimicrobial Activity

In-vitro antimicrobial activity of solvent extracts against microbial cultures of gram positive and gram negative bacteria and fungi studied were *Bacillus subtilis* (MTCC-121), *Escherchia coli* (MTCC-1652) and *Klebsiella pneumoniae* (MTCC-109) and *Aspergillus niger*. The results depict in-vitro preliminary activity in terms of the zone of inhibition in millimeter around the agar wells. The antibiotic Erythromycin (10mg/ml) was used as positive control while respective solvent were used to negative control in comparison to the plant extracts. The sample NGLS1 showed the 1mm inhibition zone against all the three bacterial strains whereas it was higher in case of sample NGLS2 i.e. 2mm in both *Bacillus Subtilis* and *E. coli*. The inhibition zone was higher for *K. pneumoniae* i.e. 5mm. However sample NGLS3 showed 1mm and 4mm zone of inhibition around the well against *Bacillus subtilis* and *K. pneumoniae* whereas *E. coli* showed resistance against this sample.

The growth of fungus *A. niger* was inhibited maximum by NGLS3. The samples of stem extracts NGLS1 show growth inhibition of all the three bacterial cultures and the zone of inhibition was found to be 3mm. NGSS2 showed zone of inhibition 3mm in both the gram negative bacteria whereas it was slightly less in gram positive i.e. 2mm. The samples NGSS3 showed maximum growth inhibition of both the gram negative bacterial culture (Fig. 1). The zone of inhibition around the agar wells was found to be somehow resistance against NGSS3. The growth of fungus *A. niger* was inhibited maximum by NGSS2, followed by NGSS1 and NGSS3 (Fig. 2). Similarly various workers have reported antimicrobial activity of various plants against various gram positive and gram negative bacterial cultures and various fungal strains viz. Ahmed Beg [11] reported that alcohol extracts of pomegranate fruits showed antibacterial activity when tested against *S.*

auxes, *E. coli* and *Shigella dysenteriae*. P. Saravanan [12] reported the solvent leaf extracts of *Achyranthus aspera* were tested for antibacterial and antifungal activities against *E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. aureus*, *Klebsiella* species.

The crude extracts of various plants viz. *Achyranthes aspera*, *A. schimperi*, *B. antidyenterica*, *P. dodecandra*, *S. incanum*, *E. caucaseum* Trautv, *E. bungei* and *A. capillus-veneris* have shown antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter species*, *Bacillus subtilis* and *Micrococcus* species, *P. vulgaris*, *S. pyogens*, *S. aureus*, *S. vmutans*, and *S. sanguis*. These plants inhibited growth of bacteria from 7 mm to >30mm diameter [13]-[15]. The results of the present study are in accordance with the studies reported earlier.

The results of the present study showed that all the extracts of stem as well as leaves showed the anti bacterial activity against *K. pneumoniae* and *Bacillus subtilis* and the bacterial cultures were found to be sensitive against all the studied extracts. The extracts of stem NGSS3 showed maximum growth inhibition of *E. coli* followed by NGSS2, NGSS1, NGSS2 and NGLS1 (Figs. 3 and 4).

VII. CONCLUSION

The observations of the present study indicate that either leaf or stem parts of the plant may be used against bacterial infection caused by *K. pneumoniae* or *E. coli* as well. The extracts of the plant may also be used against fungal diseases caused by *Aspergillus niger*.

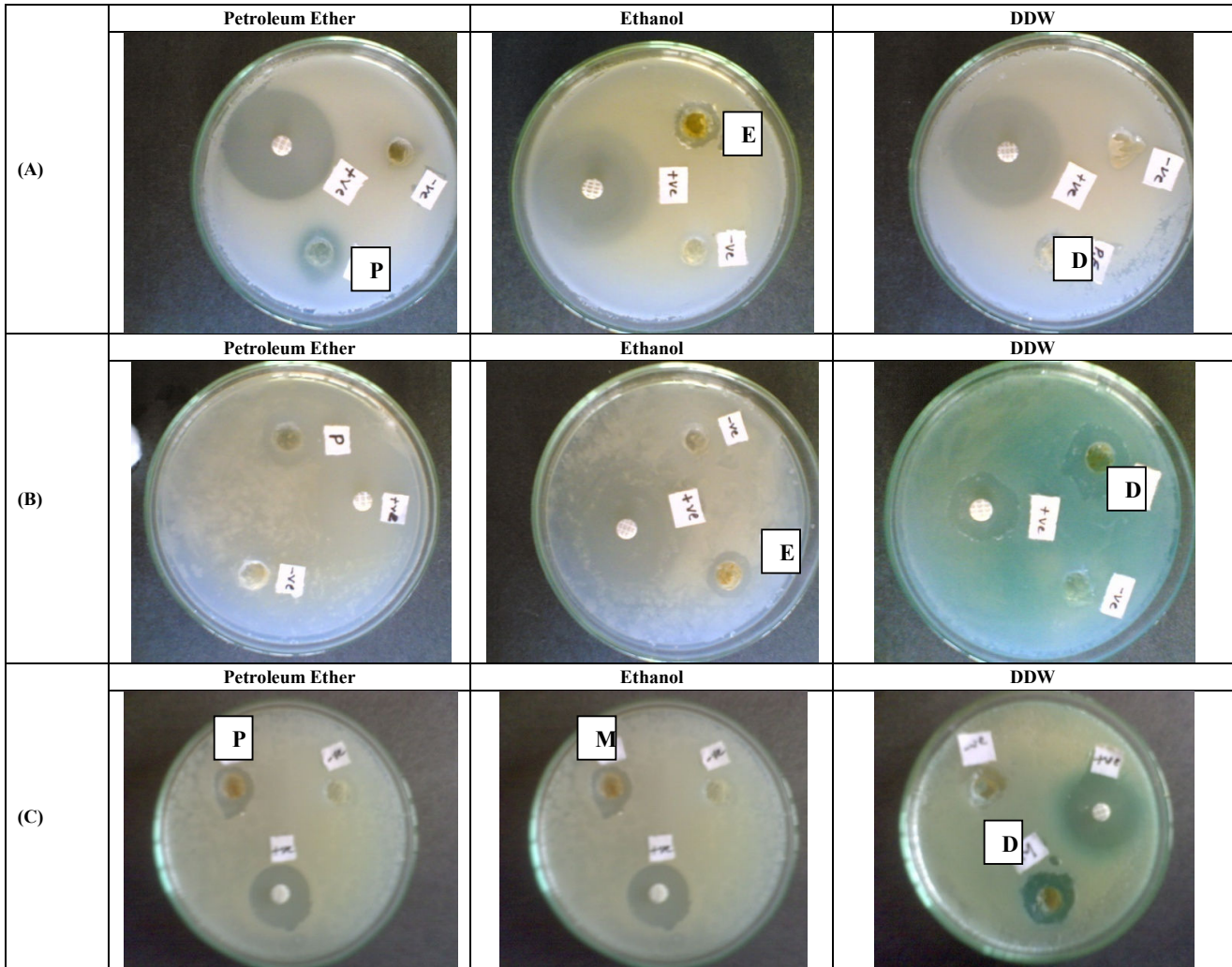


Fig. 1 (A) Antimicrobial activity of *Girardina diversifolia* Stem (A: Petroleum, B: Ethanol & C: DDW) against *Bacillus subtilis* (B) Antimicrobial activity of *Girardina diversifolia* Stem (A: Petroleum, B: Ethanol & C: DDW) against *Escherichia coli*. (C) Antimicrobial activity of *Girardina diversifolia* Stem (A: Petroleum, B: Ethanol & C: DDW) against *Klebsiella pneumoniae*

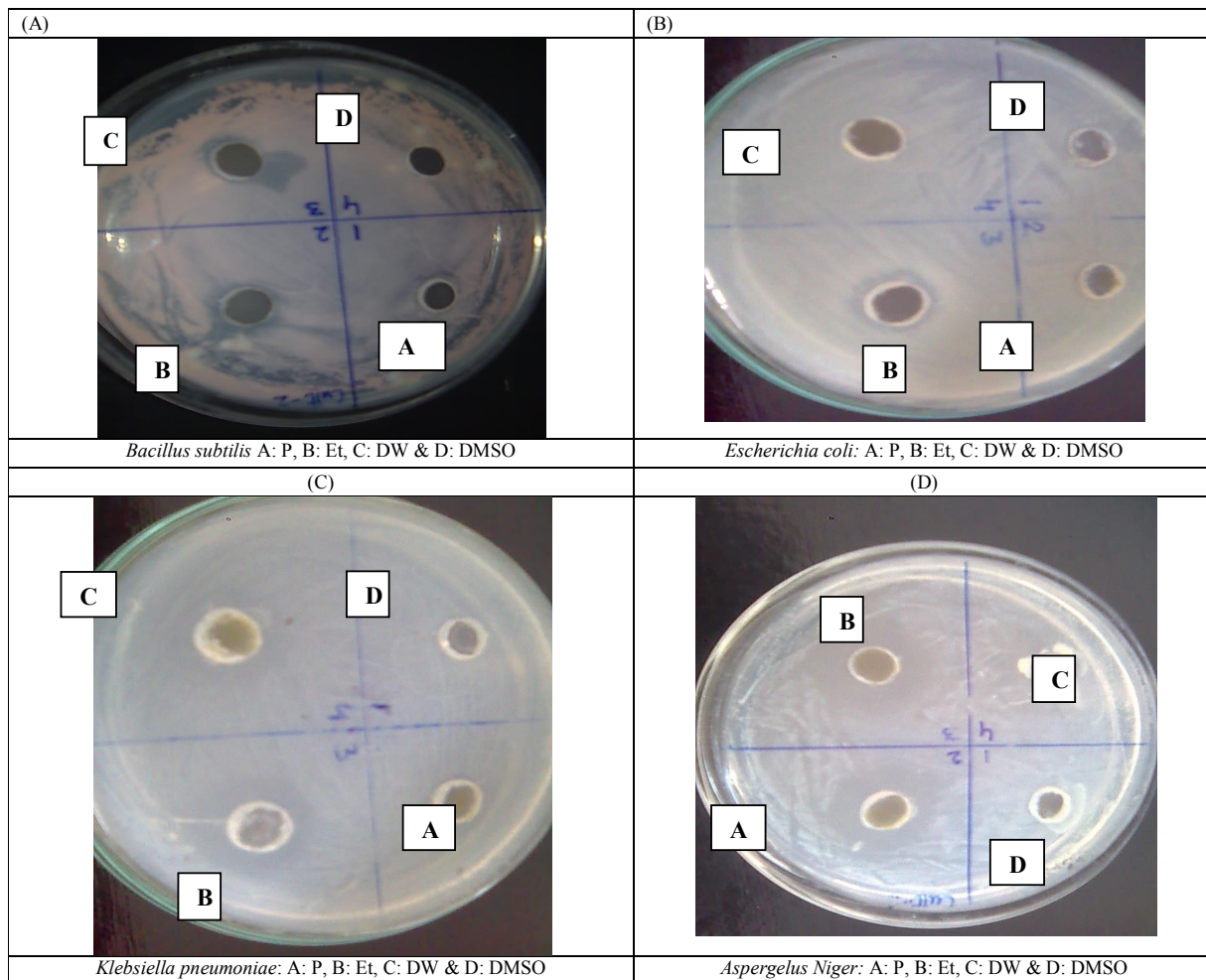


Fig. 2 (A) Antimicrobial activity of *Girardina diversifolia* leaf (Petroleum, Ethanol & DDW) against (A) *Bacillus subtilis* (B) *Escherichia coli* (C) *Klebsiella pneumoniae* & (D) *Aspergillus niger*

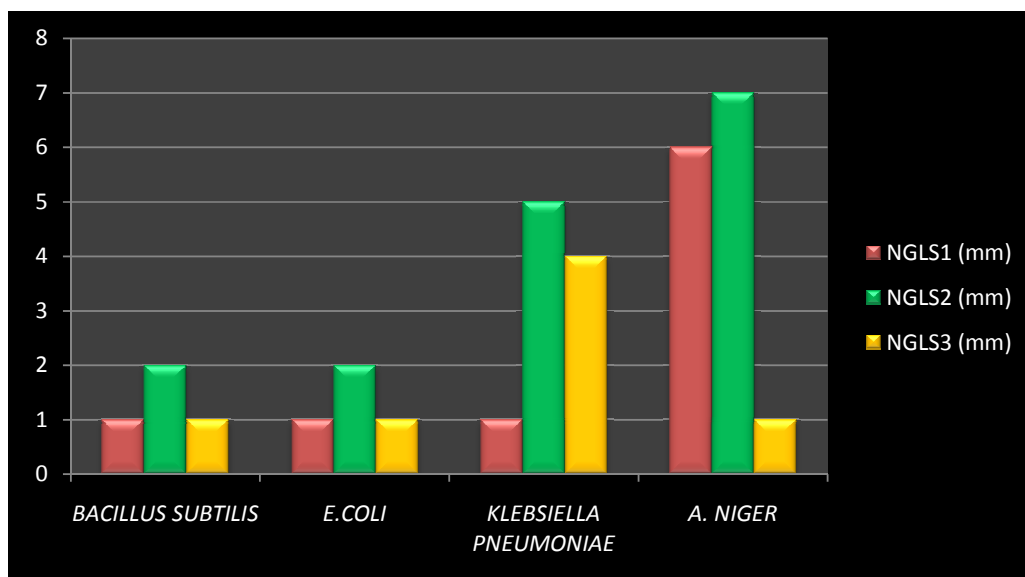


Fig. 3 Comparison between anti microbial activity of different solvent extracts of leaves

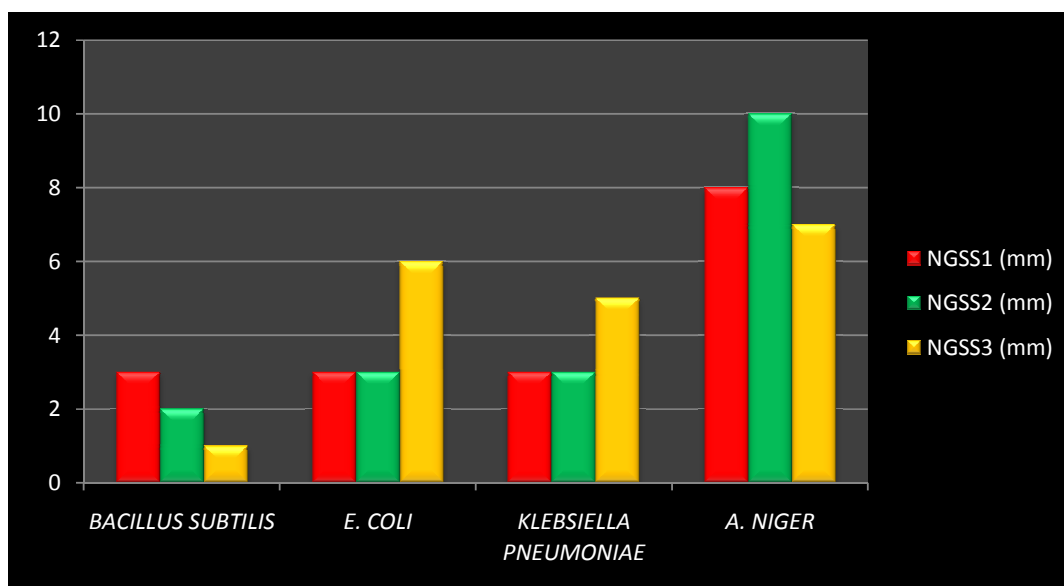


Fig. 4 Comparison between anti microbial activity of different solvent extracts of stem

ACKNOWLEDGMENT

Authors are thankful to Prof. F. C. Garg, Head Department of Microbiology of SBSPGI, Dehradun, for helping to carry out this work in the Institute and to the management of MMU, Solan for providing facilities to carry out this work.

REFERENCES

- [1] Aftab, K and A.A Sial, (1999). Book on Phyto medicine: New and old approach. *Hamdard Medicus*, 42 (2); 11-15.
- [2] Kar, D.M., B.K. Nandu, D. Pradhan, S.K. Sahu and G.K. Dash, (2004). Analgesic and antipyretic activity of fruit *Martynia annua* Linn. *Hamdard Medicus*, 47(1); 32-35.
- [3] Dhiman A and Lal, R., (2011). Phytochemical and pharmacological status. Linn. *International Journal of Research on Ayurvedic Pharmacy* 45; 224-256.
- [4] Farnsworth, N.R and D.D Soejarto., (1991). Global importance of medicinal plants. Pages 25-51 in conservation of medicinal plants.
- [5] Kamboj, V.P., (2000). Herbal medicine. *Current Science*, 95; 57-65.
- [6] Shrestha T.B. and Joshi R.M., (1987). *Rare, Endemic and Endangered Plants of Nepal*. WWF Nepal Program, Kathmandu, Nepal.
- [7] Wealth of India, (2002). A Dictionary of Indian Raw Materials a,d, Industrial Products. Publications and Information Directorate, CSIR, Hillside Rd., New Delhi, India, 3.
- [8] Per Brodal, (2010). The Central Nervous System: Structure and Function. Oxford University Press US. pp. 170-185.
- [9] Kavanagh F., (1963). Analytical Microbiology. *Academic Press London*, 4; 125-141.
- [10] Leven M, Vanner-Berghe DA, Mertens F., (1979). Medicinal Plants and its importance in antimicrobial activity. *J. Planta. Med*, 36; 311-321.
- [11] Ahmad, I. Beg, A.Z., (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants magainst multi-drug and resistant human pathogens. *Journal of Ethnopharmacol*, 74; 113-123.
- [12] P. Saravanan, V. Ramasamy, T. Shivakumar., (2008). *Asian Journal of Chemistry*, 20(1), 823- 825.
- [13] M. Manjula, V. Indira, P. Dhasarathan., (2009). Antibacterial activity of plant extracts against oral and skin pathogens; *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*, 11(2); 365-368.
- [14] Biruhalem Taye, Mirutse Giday, Abebe Animut, Jemal Seid., (2011). Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia; *Asian Pacific Journal of Tropical Biomedicine*, 3; 370-375.
- [15] Majid Alipour and Omid Khanmohammadi, (2011). *African Journal of Microbiology Research*, 5 (19); 2909-2911.