IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 1 Ver. I (Jan. 2016), PP 38-43
www.iosrjournals.org

Anti microbial Screening of Siddha Herbo-mineral medicinal formulation Kanagalinga Mezhugu against Selected Urogenital and Enteric Pathogens

Christian G J[1], Ramaswamy R S[2], Sivaraman D[3], Nijavizhi M[4], Anusha S[5]
[1](Associate Professor, Dept. of Noi Naadal, National Institute of Siddha, Chennai 47, India)
[2] (Director General, Central Council for Research in Siddha, Chennai- 106. India.)
[3](Scientist – C, Centre for Laboratory Animal Technology & Research, Sathyabama University, Chennai 119, India)
[4,5](Post graduate Scholars, National Institute of Siddha, Chennai 47, India)

Abstract: *Urogenital infections are attributed for higher rates of morbidity and mortality worldwide. Owing to the growing emergence of drug resistant strains of microorganisms to conventional antibiotics, there is a rush for the development of new drugs that are not based on synthetic antimicrobial agents for the management of recurrent urinary tract infections. The ancient Siddha system of medicine has a wealth of herbal and herbo-mineral formulations for the prevention and treatment of various gynecological conditions though the therapeutic activity responsible for the efficacy is yet unexplored much. In this present study, as a part of efficacy elucidation of Kanagalinga Mezhugu (NIS KLM) in gynecological conditions it was screened for antimicrobial activity against MTCC strains of Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Shigella sonnei, Aeromonas hydrophila, Klebsiella pneumonia, Salmonella typhimurium, Vibrio cholera, Bacillus cereus and Candida albicans. (Diploid fungus) by Hole plate diffusion method. In the screening of activity against above organisms, all organisms showed 50 % and more sensitivity to NIS KLM as compared to Ciprofloxacin control. The organisms E.coli, Pseudomonas aeruginosa, Staphylococcus aureus and Vibrio cholera showed more susceptibility to the test drug. NIS KLM also had almost equal activity against Candida albicans as compared to the standard Clotrimazole with Beclomethasone topical ointment (Candid- B^{TM}). The study therefore supports that the Siddha formulation NIS KLM used in gynecological and other disorders has a value addition of anti-microbial activity against various uro-genital infection causing organisms.*

Keywords: *Antibacterial activity, Antifungal activity, Kanagalinga Mezhugu, Siddha, Urogenital Infection.*

I. Introduction

Urogenital infections take a huge toll in terms of morbidity and related complications particularly in young, elderly and those with co-morbid conditions. The females are more affected by the above infections hence the drugs which are indicated for gynaecological problems may be of an added advantage should they exert anti microbial effects [1]. In Siddha system of medicine lot of formulations have been in use for gynaecological disorders. One such formulation is Kanagalinga Mezhugu which has a broad spectrum of indications amongst which features the gynaecological indication dysmenorrhoea [2]. The Tamil word 'Kanagam' translates into 'Gold' and 'Lingam' refers to the Ore of Mercury (Cinnabar) which being the important ingredient as other ingredients are also added in the formulation. Though Gold is not featured among the list of ingredients it has been figuratively named to mark the excellence of the formulation in treating various intractable conditions. 'Mezhugu' means a type of dosage form with waxy consistency among the 32 internal medications mentioned in the Siddha literature [3]. Therefore as a part of other gynaecological evaluations towards it, antimicrobial assaying was also done to elucidate the value addition of this test formulation against selected pathogens pertaining to urogenital tract in particular. This formulation NIS KLM as mentioned in the literature has been indicated for Arthritis, Heart disease, Dysmenorrhoea etc. There is a published clinical literature about Kanagalinga mezhugu (NIS KLM) about its efficacy on Rheumatoid Arthritis in more than 85% of patients [4]. Acute and Repeated 28 days oral toxicity study of NIS KLM conducted by the author according to OECD guidelines has found the drug to be safe in Wistar albino rats (Unpublished & Data not given here).

The *Siddha* system of medicine has its roots to the pre-antibiotic era whose enormous collection of classical literature, has in store a number of herbal, metallo-mineral, aquatic and animal products that are phenomenal in the prevention and treatment of urethral syndromes which are often caused by the infections. The test formulation was chosen based on its ability to mitigate the vitiated humours V*atham, Pitham* and *Kabam* responsible for the surfacing of signs and symptoms of disease and they are anticipated to season our body

system and render it unsuitable for the growth of micro organisms causing disease. The presence of various bioactive chemicals in plant derived drugs is expected to have systemic action on gynaecological ailments which includes uterine relaxant, hormonal regulatory and thereby anti-dysmenorrheal properties. Though this formulation is claimed as an antibiotic, its antimicrobial activity needs to be screened and substantially validated. In this article an attempt was made to screen the antibacterial and antifungal potential of the *Siddha* formulation NIS KLM that is mentioned in the Siddha text *Anuboga Vaithya Navaneetham* and being commonly followed by traditional *Siddha* practitioners in the management of menstrual irregularities.

II. Detailed information of Kanagalinga Mezhugu (NIS KLM)

2.1 Siddha method of purification of selected Ingredients: [3]

2.1.1 *Lingam (Mercuric Sulphide)* : Soaked in Lemon juice for 9 hours.

2.1.2 *Veeram (Mercuric perchloride)*: Covered in a cotton cloth and hung above the mixture of Tropical amaranth (Amaranthus polygonoides) inside a vessel and heated well.

2.1.3 *Pooram (Mercuric subchloride)*: Covered in a cotton cloth and dipped inside coconut water containing the dissolved paste of Betel leaves and pepper before heating it to a rolling boil.

2.1.4 *Rasam (Mercury)*: Sublimated from Lingam (Cinnabar-Mercuric sulphide) by heating it inside an earthenware pot.

2.1.5 *Thalagam (Arsenic trisulphide)*: Seasoning in slaked lime water.

2.1.6 *Gandhagam (Sulphur)*: Melting the sulphur and pouring into Henna Curd Mixture 7 times.

The ingredients mentioned in Table 1 were ground in a motorized stone mortar for 12 hours with Honey, Onion juice, Garlic juice, Anda thailam and castor oil added together[4]. Anda thailam is a traditional Siddha oil formulation processed by searing the hens' egg yolk in a pan until it chars and oil is exuded and expressed. [3] Anda thailam is added to detoxify the negative effects of mercurials that constitute 25% of the total formulation as per the traditional experience [3,4&5] . The formulation was potentiated by adding certain ingredients mentioned in other formulations of Anuboga Vaithya navaneetham with same indications to increase the efficacy of the Trial drug as approved by the IAEC and IEC of National Institute of Siddha [2]. And the formulation was proved to be non toxic in Repeated sub acute oral toxicity study performed according to OECD 423 and 407 guidelines. The trial drug NIS KLM was stored in a cool and dry place within a clean and dry porcelain vessel.

Objective

The aim of the present study is to screen the antimicrobial potential of the *Siddha* formulation Kanagalinga Karpoora Mezhugu (NIS KLM) against selected Uropathogens.

III. Materials and methods

The ingredients of this herbo-mineral *Siddha* formulation were market samples procured from country medicine shop at Broadway,Chennai. The test formulation had herbal, mineral, metallic and marine compounds as per the *Siddha* literature [2].

3.1 Test Procedure

The antimicrobial study was conducted at Centre for Laboratory Animal Technology and Research, Sathyabama University, Chennai, Tamilnadu, India.

3.2 Cleaning and Sterilization

The Glasswares used in the present study were cleaned with cleaning solution and sterilized in hot air oven to 180^0C for 3 hours. All nutrient media were sterilized by autoclave (121^0C, 15psi for 15-20 mins).

3.3 Culture of Pathogens

The Microbial strains used in the sensitivity assay were *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 432), *Proteus mirabilis* (MTCC 3310), *Staphylococcus aureus* (MTCC 737) , *Pseudomonas aeruginosa* (MTCC 424) , *Shigella sonnei* (MTCC 646), *Aeromonas hydrophila* (MTCC 1739), *Salmonella typhimurium* (MTCC 733), *Vibrio cholera* (MTCC 3906), *Bacillus cereus* (MTCC 430) and *Candida albicans. (Diploid fungus) (MTCC 854)* were purchased from MTCC, Chandigarh , India and they were sub cultured as per the guideline and standard protocol laid down by National committee for clinical Laboratory standards. Microbial Stock cultures were maintained at 4°C on slopes of nutrient agar. (Hi Media, Mumbai **)**

Heavy metallic salts	**Non Metallic Mineral salts**	**Herbal exudates (Gums & Resins)**	**Herbs**
Lingam (Mercuric sulphide) 1000mg	Ganthagam(Sulphur) 320mg	Palingu Saambirani (White Frankincense) 640mg	Nervalam(Croton tigilium) 500mg
Veeram (Mercuric perchloride) 800mg	Navachaaram (Ammonium chloride) 320mg	Perungayam (Asafoetida) 640mg	Saadhikkai (Nutmeg) 160mg
Pooram (Mercuric subchloride) 1000mg	Palagarai Parpam (Cowrie Calcinate) 500mg	Soodan(Camphor) 640mg	Karbogi arisi (Psoralea corylifolia) 160mg
Rasam (Elemental mercury) 320mg	Puneeru (Fullersearth) 500mg	Pachai Karpooram (Edible camphor) 160mg	Peeled sukku (Zingiber officinale) 160mg
Thaalagam (Arsenic trisulphide) 150mg	Vediuppu (Pottasium nitrate) 320mg Induppu (Rock salt) 320mg Vengaram (Borax) 320mg		Milagu (Piper nigrum) 160mg Thippili *(Long Pepper)* 160mg Koshtam *(Saussurea lappa)* 160mg Sitrarathai *(Alpinia calcarata)* 160mg Thesaavaram (*Piper longum* root) 160mg Sithira moolam root bark *(Plumbago zeylanica) 160mg* Akkarakaram *(Spilanthes acmella)* 160mg Sanninayagam 160mg Omam (Carum copticum) 160mg

Table 1. Ingredients of Kanagalinga Mezhugu (NIS KLM)

3.4 Preparation of Test Drug sample

Test sample (NIS KLM) provided for evaluation was colloidal in nature and in order to make the sample less viscous and suitable for handling through pipette about 2 ml of sterile distilled was added to make up to the volume and triturated for nearly 30 minutes to make up a homogenous liquid.

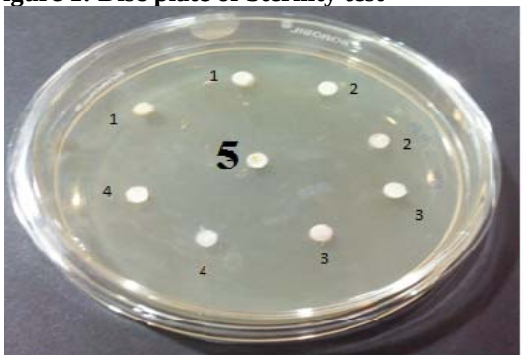
3.5 Standard control

Standard drug was used as control in this study. Ciprofloxacin was used for anti-bacterial study and the marketed formulation Candid –B^{TM} cream (clotrimazole-betamethasone) was used for anti-fungal study.

3.6 Sterility Test for Test drug NIS KLM

The test formulation was subjected to the preliminary sterility evaluation by disc plate method. Freshly prepared nutrient agar medium was loaded on the sterile disc and the same was used for enumeration of sterility of the test formulation. As the test formulation is colloidal in nature streaking or swabbing is not possible. Diluted form of test formulation at the concentration of 200 µl was loaded on to the top of the disc and incubated for the period of 48 hours with timely observation in between. Incubated plate was observed for 12, 24 and 48 hours after incubation and no growth of organism either as an isolated or as a colony was found in the incubated formulation. (Fig. 1)

Figure 1: Disc plate of Sterility test



Disc 5 – NIS KLM, Evaluated at single concentration of 200 µl *(Other discs not relevant here)*

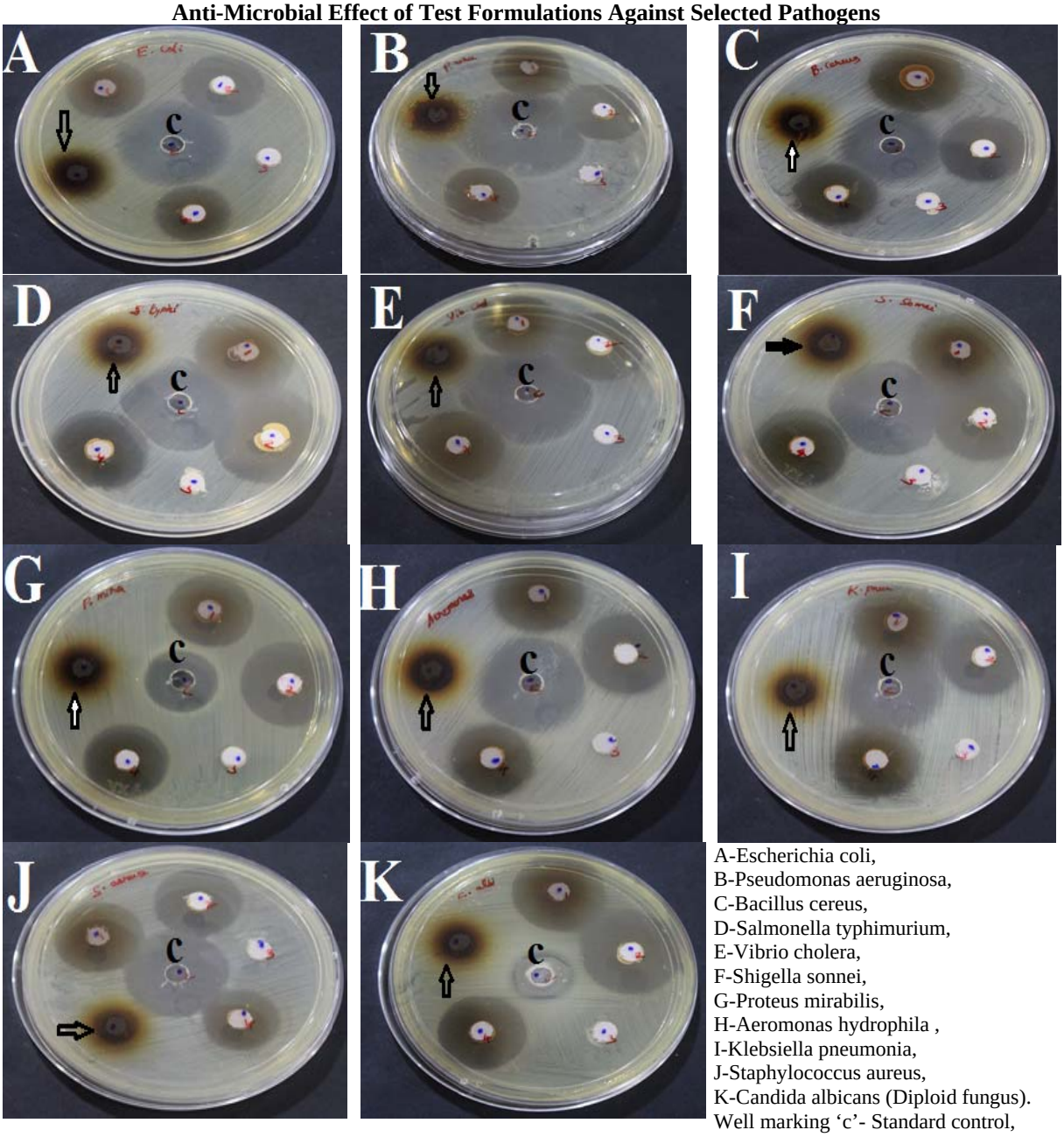
3.7 Preparation of inoculums

Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria and $2.0 \cdot 10^5$ spore/ml for fungal strains [9].

3.8 Hole-plate diffusion method [7,8,10] : Equidistant holes of 6mm were made in the agar using sterile cork borers. A 100µL volume of sample solution was added to the holes using a pipette or (Eppendorf). The compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The same procedure was followed for the fungus also. These studies were performed in triplicate.

IV. Results and Discussion

Anti-Microbial Effect of Test Formulations Against Selected Pathogens



A-Escherichia coli,
B-Pseudomonas aeruginosa,
C-Bacillus cereus,
D-Salmonella typhimurium,
E-Vibrio cholera,
F-Shigella sonnei,
G-Proteus mirabilis,
H-Aeromonas hydrophila ,
I-Klebsiella pneumonia,
J-Staphylococcus aureus,
K-Candida albicans (Diploid fungus).
Well marking 'c'- Standard control,
Arrows show Well markings of NIS KLM.

Table 2. Zone of Inhibition in mm and Percentage as compared with Standard drugs

The value represented in the table is a triplicate performed for each formulation against each organism. The zone diameter mentioned as a range of minimum to maximum values in mm.

S.No	Test Organism	Inhibition Zone Diameter in mm		Corresponding % of NIS KLM-inhibition zone with Standard
		Standard Control (Bacteria) Ciprofloxacin	NIS KLM	
1	*Escherichia coli*	29-32	17-22	59-69
2	*Pseudomonas aeruginosa*	29-33	14-17	48-52
3	*Proteus mirabilis*	19-22	9-11	47-50
4	*Staphylococcus aureus*	28-31	15-19	53-61
5	*Shigella sonnei*	25-29	12-16	48-55
6	*Aeromonas hydrophila*	25-27	11-15	44-55
7	*Klebsiella pneumonia*	26-30	11-14	42-47
8	*Salmonella typhimurium*	29-33	13-17	45-52
9	*Vibrio cholerae*	30-34	14-19	47-56
10	*Bacillus cereus*	24-28	8-12	33-43
11	*Candida albicans*	14-17 (Clotrimazole)	12-15	86-88

4.1 Antimicrobial activity

The *Siddha* formulation, *Kanagalinga Mezhugu*(NIS KLM) showed moderate broad spectrum antimicrobial activity against gram-negative and gram positive organisms (Fig A-J) and Table 2 of which the organism *E.coli* showed a higher zone of susceptibility approx. 70% against the standard positive control Ciprofloxacin (Ranbaxy). Also the test drug was approximately 50% as sensitive as that of ciprofloxacin against other organisms listed in the Table 2. The test drug NIS KLM had very good sensitivity of around 88% as that of Clotrimazole and Beclomethasone (Candid - B^{TM}) against *Candida albicans* a fungus. Therefore this drug when administered for gynaecological problems can have an additional advantage of antimicrobial efficacy which would be useful in the treatment of Pelvic Inflammatory diseases also.

The drug *Kanagalinga Mezhugu* is an age-old and celebrated herbo-mineral *Siddha* formulation and has been indicated in *Siddha* literature for various kind of diseases which include gynaecological ailments like dysmenorrhoea and mass in the uterus [2]. The ingredients mercury, Sulphur and others (Table 1) were purified as per *Siddha* classical literature and they were transformed into absorbable non-toxic compounds that are therapeutically effective. The triturating of metals ores with herbal juices along with other herbal ingredients and grinding for longer duration diminishes and nullifies the toxicity and causes the reduction in particle size and they are transformed to nano particles due to the alteration of their chemical structure (Austin et al., 2012). *Siddha* formulations which contain Mercury and sulphur have these two combined to form Mercuric sulphide (HgS) which is the least toxic and less absorbable mercurial compound than any other. (Hardy et al.,1995). Cinnabar (HgS) or 'lingam' in Tamil parlance is being used in Siddha medicine for many years.

V. Conclusion

Our findings suggest that *Kanagalinga mezhugu* (NIS KLM) shows broad spectral antibacterial activity against the tested organisms of which *Escherichia coli* had significant sensitivity against the test drug. Hence it can be acknowledged as a potential drug with antimicrobial sensitivity against these common and Urinary tract pathogens and would be a worthwhile agent in the management of Gynaecological disorders. More researches may be warranted at the molecular level and also the pharmacodynamic targets of these age old *Siddha* medicines should be identified to optimize clinical success in the management and prevention of urinary infections.

VI. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement.

This study was carried out with the guidance of Staff of Centre for Laboratory Animal Technology and Reseach, Sathyabama University, Chennai, Tamilnadu, India.

References

[1]. Dennis L.Kasper, Anthony S.Fauci, Stephen L.Hauser, Dan L.Longo, Larry Jameson J, Joseph Loscalzo. Harrison's Principles of Internal Medicine, 19th edition, 2015: 861.
[2]. P.M Abdullah Saheb, Anuboga vaidhya navaneetham,Dec,2001,S.P Ramachandran,Thamarai Noolagam,Vadapalani,Chennai-26. Vol:4 Page 24 Kanaga linga mezhugu; Veera mezhugu Vol 4 139; Anda thylam Vol 4 21,144.
[3]. Thiagarajan R, Gunapadam-Thathu Jeeva vaguppu, 4th edition,2004; Directorate of Indian
[4]. Medicine & Homeopathy,Govt.of Tamil Nadu

[5]. Eleza C, Sivanandan S. Efficacy of Kanagalinga Karpoora mezhugu on Rheumatoid Arthritis. Indian Medicine and Homeopathy Journal, 2002 Jul-Sep: 20
[6]. Rai Bahadur Jaisingh P Modi. A Text book of Medical Jurisprudence and Toxicology, Elsevier, 2013 Oct: P. 580
[7]. Anoop Austin, 2012, Chemical characterization of gold and mercury based Siddha sasthric preparation. American journal of drug discovery and development.2(3):110-123
[8]. Sahin F M, Daferera D, SO kmen, A., SO kmen M, Polissiou M. Biological activities of the essential oils and methanol extract of Origanum vulgare ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control.2004;15: 549–557.
[9]. F. Sharififar , M.H Moshafi . In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora BoissFood Control.2007; 18: 800–805.
[10]. Varaprasad Bobbarala, Prasanth Kumar Katikala , K. Chandrasekhar Naidu and Somasekhar Penumajji. Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger F2723. Indian Journal of Science and Technology. 2009;2 (4):87-90.
[11]. Perez C and Paul M and Bezique P (1990) An Antibiotic assay by the agar well diffusion method. Alta Biomed. Group Experiences. 1990;15:113-125.
[12]. Maryam Afsharzadeh, Mahboobe Naderinasab, Invitro Antimicrobial activities of some Iranian conifers, Iranian Journal of Pharmaceutical Research. IJPR 2013; 12(1), 63-74.
[13]. Janifer J, Geethalakshmi JS, Satyavani K, and Viswanathan V. Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol. 2009 Jul; 19(3): 107–111
[14]. Bang RA, Bang AT, Baitule M, Choudhary Y, Sarmukaddam S, Tale O. High prevalence of gynaecological diseases in rural Indian women. Lancet 1989; 1:85-8.
[15]. Rustam et al., 2010,Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future.Front Microbiol. 1: 134.
[16]. Chopra I et al., 2002,"Discovery and development of new anti-bacterial drugs," in Pharmacochemistry Library,. vol 32, Trends in Drug Research III, ed. van der Goot H., editor. (Amsterdam: Elsevier;), 213–225
[17]. Hasan et al.,2006,Evaluation of antibacterial Activity and phytochemical analysis of root extract of Boscia Angustifolia. African Journal of biotechnology, 5(18); pp 1602-1607.
[18]. Hardy.A.D, H.H.Sutherland, R.Vaishnav and M.A. Worthing, 1995, report onn the composition of mercurials used in traditional medicines in Oman. ,J.Ethnopharmacol.49;17-22.
[19]. Samuel Baron. Purushothama V. Dasaraju and Chien Liu , 1996,Medical Microbiology. 4th edition. (TX): University Galveston of Texas Medical Branch at Galveston.