Antibacterial Activity of Majoon-e-Maddat-ul-Hayat Jadwari Against Uropathogenic *Escherichia coli* (UPEC)

[1]*Meera Devi Sri P, [1]Rampratap Meena, [1]D. Ramasamy, [1]S. Mageswari, [2]Shamsul Arfin, [2]Aminuddin and [1]Rafath Mehmooda

[1]Regional Research Institute of Unani Medicine,
No. 1 West Madha Church Street,
Royapuram, Chennai-600013

[2]Central Council for Research in Unani Medicine,
61-65 Institutional Area,
Janakpuri, New Delhi-110058

Abstract

The present study was carried out to evaluate the antibacterial activity and to compare the potency of the drug Majoon-e-Maddat-ul-Hayat Jadwari against the uropathogenic strains of *Escherichia coli* isolated from the urine samples of diabetic mellitus patients and pregnant women. Majoon-e-Maddat-ul-Hayat Jadwari, a polyherbal Unani formulation was prepared at laboratory scale and assessed for its potency against the clinical strains of uropathogenic *Escherichia coli* using the cup plate method. The Minimum Inhibitory Concentration (MIC) of the drug was also determined using agar diffusion method. Different strains exhibited different mode of sensitivity against the drug. On comparison, the *E.coli* isolated from the urine sample of pregnant women exhibited more sensitiveness to the drug than the *E.coli* isolated from the urine of diabetic mellitus patients. Some strains of *E.coli* irrespective of their source, did not exhibit any activity. The MIC of the drug was found to be within the range from 150 mg/ml to 200mg/ml in case of *E.coli* strains isolated from the urine of diabetes mellitus patients and 75mg/ml to 200mg/ml against the *E.coli* strains from pregnant women.

Key words: Majoon-e-Maddat-ul-Hayat Jadwari, *Escherichia coli,* Antimicrobial activity.

Introduction

Urinary tract infection (UTI) is one of the most important complications encountered by many people worldwide and is the active infection in any part of urinary tract beyond the distal urethra which is normally sterile. The chances of urinary tract infection are more common in cases of women than men with predominance to diabetes mellitus patients and pregnant women (Chen *et al.,* 2009; Duarte *et al.,* 2008). Urinary tract infection in both diabetes mellitus patients and pregnant women are asymptomatic, if unrecognized or untreated properly may lead to several complications (Balachandar *et al.,* 2002). UTI in pregnant women may be due to factors like physical and hormonal changes during the pregnancy. The increased level of progesterone during pregnancy causes the muscles that line the urine making urethras to relax and allow the bacteria to rise up into the bladder making it susceptible to infection, the enlarged uterus also prevent the complete emptying of the bladder by leaving a pool of urine which acts as a good source for multiplication of bacteria. Additionally, the physiologic increase in plasma volume during pregnancy decreases the urine concentration and leads to UTI. It is also estimated that up to 70 percent of pregnant women develops glycosuria, which encourages

[1]* Author for correspondence

bacterial growth in the urine (Saleem and Daniel, 2011). Increased level of urinary progestins and estrogens also leads to decreased ability of the lower urinary tract to resist invading bacteria possibly allowing some strains of bacteria to selectively grow in this environment.

In patients of diabetes mellitus, urinary tract infections (UTIs) is very common because of hyperglycemia (high blood sugar), where the increased sugar in the urine acts as a friendly environment for the bacterial cultures to grow. The other factors like age, poor metabolic control, duration of DM, defects in neutrophil function, frequent hospitalization, recurrent vaginitis, and vascular complications also play a major role in the high incidence of UTI in diabetic patients.

The major etiological agent of UTI in both the cases falls in the Enterobacteriaceae family of which nearly 80% of infection occurs mainly due to *Escherichia coli* (Ronald, 2002). Though the organism are usually the commensals of the intestines some uropathogenic strains of *Escherichia coli* (UPEC's) causes wide range of UTI's, leading to urethritis/cystitis, symptomatic cystitis, pyelonephritis, acute prostatitis, prostatic abscess, and urosepsis together with general symptoms including (i) pressure in the lower pelvis (ii) painful urination (Dysuria) (iii) frequent urination (Polyuria) or urgent need to urinate (Urinary urgency) (iv) need to urinate at night (Nocturia) (v) urine that contains traces of blood (Hematuria) (vi) dark, cloudy or strong-smelling urine (vii) pain above the pubic bone, or in the lower back or abdomen (viii) feeling unwell, weak or feverish (ix) very frequent urge to urinate and weak feeling of head because of tiredness caused by dehydration (x) intense desire to pass more urine after urination (Stangury)

Among the Indian systems of medicines, Unani system of medicine gained much popularity by producing a variety of efficient and safe therapeutic agents in various forms of formulations for several disorders. Majoon-e-Maddat-ul-Hayat Jadwari is one such polyherbal formulation used in the treatment of ailments like Salasul Baul (Incontinence of urine), Waj-ul-Kulya (pain in the kidney), Waj-ul-Qutn (Pain in the Lumbar) and Waj-ul-Masana (pain in the bladder) which can be correlated with the general signs and symptoms of the UTI's.

With this view, the present study was designed, to study and to compare the efficacy of the drug Majoon-e-Maddat-ul-Hayat Jadwari against various strains of *Escherichia coli* isolated from the urine samples of diabetes mellitus patients and pregnant women.

Materials and Methods

The formulation Majoon-e-Maddat-ul-Hayat Jadwari (Anonymous, 2008) was prepared at lab scale in Drug Standardisation Research Unit (DSRU) of RRIUM,

Chennai for the development of Standard Operating Procedures (SOP) and to evaluate pharmacopoeial standards. All the drugs were procured from R.N. Rajan & Company, Chennai. The drug was prepared using the authenticated ingredients namely Filfil Siyah (*Piper nigrum* Linn. DSM-44) Fruit, Filfil Daraz (*Piper longum* Linn. DSM-45) Fruit, Darchini (*Cinnamomum zeylanicum* Blume. DSM-40) Inner stem bark, Jadwar Khatai (*Delphinium denudatum* Wall. DSM-131) Root, Ood Saleeb (*Paeonia emodi* Wall. DSM-57) Tuber, Behman Surkh (*Salvia haematoides* Linn DSM-36) Root, Aamla (*Emblica officinalis* Gaertn. DSM-7) Fruit, Post-e-Balela (*Terminalia belerica* Roxb. DSM-56) Fruit, Sheetraj hindi (*Plumbago zeylanica* Linn. DSM-138) Root, Zarawand (*Aristolochia indica* Linn. DSM-124) Root, Gul-e-Babuna (*Matricaria chamomilla* Linn. DSM-26) Flower, Khusyat-us-Salab (*Orchis latifolia* Linn. DSM-58) Tuber, Chilghoza (*Pinus gerardiana* Wall. DSM-100) Seed, Maghz-e-Narjeel (*Cocos nucifera* Linn. DSM-129) Endosperm, Arq-e-Babuna (*Matricaria chamomilla* Linn DSM-26) and Maweez Munaqqa (*Vitis vinifera* Linn. DSM-139) Distillate (Anonymous, 2008) .

Collection of microorganism

A Total of fifteen urine samples (eight from the Diabetes mellitus patients and seven from pregnant women) suspected for urinary tract infection (significant bacteriuria >100,00 col/ml) were collected from various clinical laboratories in Chennai (Excellent laboratory, Madras diagnostic centers & Olympus laboratory). All the samples were subjected to conventional microbiological analysis using Macconkey agar and Blood agar (Mackie & McCartney, 1996). Bacteria suspected for *Escherichia coli* were alone subjected to further Biochemical tests (IMViC). Pure cultures of six different isolates of *Escherichia coli* from the urine sample of Diabetes mellitus patients coded (ECODM-I, ECODM-II, ECODM-III, ECODM-IV, ECODM-V & ECODM-VI) and five different isolates of *Escherichia coli* from the urine samples of pregnant women coded (ECOPW-VII, ECOPW-VIII, ECOPW-IX, ECOPW-X & ECOPW-XI) were stored in the nutrient agar slants for further analysis.

Antibacterial activity

The invitro antibacterial susceptibility test was performed using the cup plate method (Divakar, *et al.,* 2001). The required number of muller hinton agar plates were prepared and swabbed with different isolates of lag phase cultures of *Escherichia coli* isolated from the urine samples of both diabetes mellitus patients and pregnant women. The plates were allowed to stand for few minutes. Required numbers of 6mm diameter wells were made over the plates at an equidistant position. Wells were loaded with 50µl of the drug at

the concentration of 300mg/ml. Dimethylsulphoxide (DMSO) was used as the solvent. Separate control disc was also included using the solvent. The ciprofloxacin (250mg/ml) dissolved in sterile DMSO was used as standard for comparison (Anonymous 1982). The bacterial plates were kept at 37°C for 18-24 hours. The zone of inhibition was measured using the calipers.

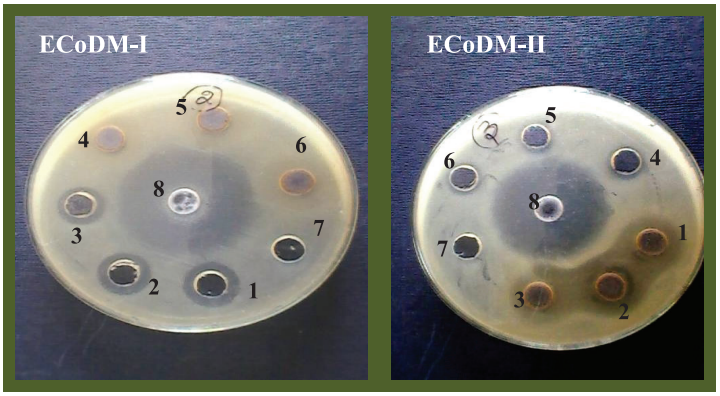
Minimum inhibitory concentration (MIC)

The MIC, the lowest concentration of the drug required to inhibit the microorganism was also determined by the agar dilution method (Anonymous, 1982) using the different concentration (300mg/ml, 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) of the drug. The lowest concentration of the drug (MIC) that completely inhibits the growth was determined after overnight incubation at 37°C.

Table 1

S. No.	Microorganisms	Zone diameter in mm (n=2)							Std (Cipr) 250 mg/ml
		300 mg/ml	250 mg/ml	200 mg/ml	150 mg/ml	100 mg/ml	75 mg/ml	50 mg/ml	
1	Escherichia coli (ECoDM-I)	17	15	11	8	NA	NA	NA	S
2	Escherichia coli (ECoDM-II)	20	18	15	9	NA	NA	NA	S
3	Escherichia coli (ECoDM-III)	NA	NA	NA	NA	NA	NA	NA	R
4	Escherichia coli (ECoDM-IV)	19	17	16	10	8	NA	NA	S
5	Escherichia coli (ECoDM-V)	18	15	11	9	NA	NA	NA	R
6	Escherichia coli (ECoDM-VI)	17	16	14	11	8	NA	NA	S
7	Escherichia coli (ATCC 25922)	20	19	18	15	10	9	7	S
8	Escherichia coli (ECoPW-VII)	22	21	18	15	10	7	NA	S
9	Escherichia coli (ECoPW-VIII)	21	20	18	17	15	8	NA	S
10	Escherichia coli (ECoPW-IX)	NA	NA	NA	NA	NA	NA	NA	R
11	Escherichia coli (ECoPW-X)	19	18	15	13	8	NA	NA	S
12	Escherichia coli (ECoPW-XI)	18	17	14	12	7	NA	NA	S
S – Sensitiveness ; R – Resistant ; Cipr – Ciprofloxacin. NA – No Activity									

Fig.1: Antibacterial activity of ECoDM and ECoPW



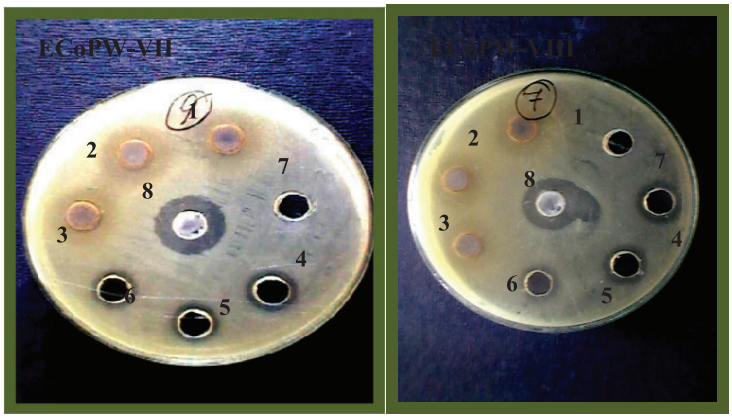
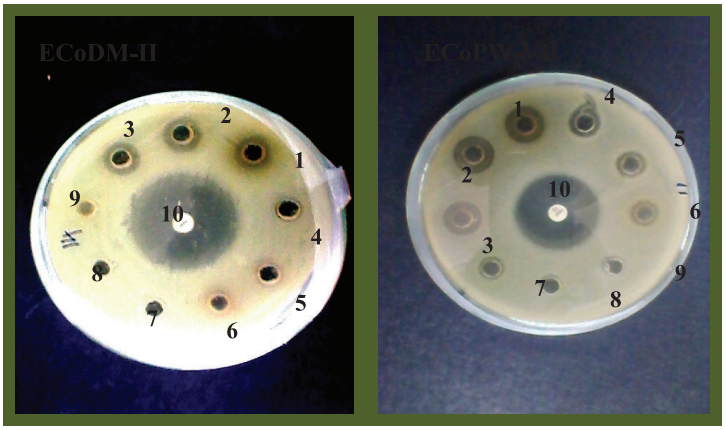


Fig. 2: Comparison of Escherichia coli (ECoDM & ECoPW)



Results and Discussion

A total of eleven different strains of *Escherichia coli* were isolated and confirmed from urine samples (seven from diabetes mellitus patients and five from pregnant women). Different strains exhibited different mode of sensitivity

against the drug. In general uropathogenic strains of E. coli is expected to have an adherence factor called P fimbriae, or pili. These P fimbriae mediate the attachment of E. coli to uroepithelial cells. Thus, patients with intestinal carriage of E. coli that contains P fimbriae are at greater risk of developing UTI than the general population. On comparison the drug exhibited good activity against the *E.coli* isolated from the urine of pregnant women than the *E.coli* isolated from the urine of diabetic mellitus patients. Some strains of *E.coli* irrespective of their source does not show any activity. The MIC of the drug was found to be in the range of 150 mg/ml to 200mg/ml in case of *E.coli* strains isolated from the urine of diabetes mellitus patients and 75mg/ml to 100mg/ml against the *E.coli* strains from pregnant women (Table – 1; Fig- 1 & 2).

Conclusion

On conclusion the different degree of virulence can be correlated with the genetic makeup of the strains and further investigation on molecular level may aid in the discovery and development of new drugs in future to encounter the human uropathogenic *Escherichia coli* (UPEC).

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