Studies on Physico-chemical Standardization and Antibacterial Activity of 'Jawarish-e-Zanjabeel' – A Unani Polyherbal Formulation

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Abstract

The compound drug Jawarish-e-Zanjabeel is a poly herbal Unani drug. It is being used in the various ailments like diarrhea, anorexia and flatulence in the stomach. The present study was designed to evaluate the pharmacopoeial standards, WHO parameters, antibacterial activity and MIC of the drug. The quality control parameters like microbial content, heavy metals, aflatoxin and pesticidial residues were found within permissible limits. The antimicrobial study revealed that the drug has potent activity against the uropathogenic organisms responsible for urinary infections like uretritis, cystitis, pyelonepritis, acute prostatitis and urosepsis. On comparison the drug exhibited higher degree of activity against all the the *E.coli* strains whereas moderate to low activity against the other tested organisms at 50 mg/ml concentration.

Key words: Physico-chemical parameters, TLC, Heavy metals, Microbial load, Aflatoxins, Pesticide residues, Antibacterial activity.

1. Introduction

In modern era, herbal medicines are seen as potential medicines for a variety of diseases. There has been a striking increase in use of traditional system of medicine in both developing and developed countries due to their natural origin and no side effects (Pulok Mukherjee, 2008). But challenges are many before realizing the dream of safe and potent drug for effective treatment of patients and improvement of quality of life. There remains a challenge for developing a scientific basis of herbal medicines. Therefore, standardization and clinical activities are highly warned. However, the advent of quality standardization and biological activities of these drugs will certainly open new frontiers for treatment of many diseases (Sharma and Arora, 2006). The drug Jawarish-e-Zanjabeel is one of the poly herbal Unani formulation categorized under Majooniath listed in the National Formulary of Unani Medicine, Part - I. The drug was prepared in three batches at laboratory scale using authenticated raw drugs as per Standard Operating Procedure (SOP). The drug is being used in the ailments of diarrhea, anorexia and flatulence in the stomach (Anonymous, 2006).

The urinary tract infection is the most common infection affecting the urinary systems- kidney, ureteres, blader and urethra. Normally urine is sterile and is free of microbes. Infections occurs when micro organisms especially bacteria like *E.coli*, *Klebsiella* species, *Staphylococcus* species enters in the urinary systems.

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Hence, the present study was subjected to evaluate physico-chemical parameters, thin layer chromatography, heavy metal, microbial contamination, aflatoxin level, pesticide residue and antibacterial activity to assess the potent activity of the drug against the UTI pathogens isolated from the UTI patients.

2. Material and Methods

2.1. Collection of Raw drugs

In order to develop a scientific method for the preparation of this formulation, the raw drugs were procured from local raw drug dealers Chennai. All the raw drugs were identified by botanist using pharmacognostical methods (Kokate, 2000). Jawarish-e-Zanjabeel drug was prepared as per the formulation composition given in NFUM part–I (Anonymous, 2006). The formulation contains eight single drugs namely Zanjabeel (*Zingiber officinale* Rosc. – Rhizome), Samagh-e-Arabi (*Acacia latifolia* (L) Willd.ex.Del – Gum), Dana Heel Khurd (*Elettaria cardamomum* (L) Maton. – Seed), Belgiri (*Aegle marmelos* Corr. - Fruit pulp), Saleekha (*Cinnamomum cassia* Blume. - Stem bark), Zarambad (*Curcuma zedoaria* Rosc. – Rhizome), Nishashta-e-Gandum (*Triticum aestivum* Linn. - Starch powder) and Sugar.

2.2. Collection of microorganism

Urine samples of 25 UTI infected patients were collected from various hospitals and clinical laboratories in Chennai. All the samples were subjected to conventional microbiological analysis using Macconkey agar and Blood agar (Mackie & McCartney, 1996). The pure cultures of three strains of *E.coli* coded as JZECO-1, JZECO-2, JZECO-3 , three strains of *Klebsiella pneumoniae* coded as JZKP-1, JZKP-2, JZKP-3 and three strains of *Staphylococcus saprophyticus* coded as JZSS-1, JZSS-2, JZSS-3 were maintained in nutrient agar slants and were used for further studies. All the cultures were confirmed at the molecular level in the Department of Microbiology and compared with the NCBI database.

2.3. Physico-chemical analysis

The prepared three batch samples were subjected for Physico-chemical studies like total ash, acid insoluble ash, water soluble ash, solubility in alcohol and water, loss on drying at 105º. The bulk density, sugar estimation and *pH* values for 1% and 10% aqueous solution were also carried out (Anonymous, 1987).

2.4. Thin layer chromatography

The chloroform and alcohol extracts of the drug were applied on precoated silica gel 60 F_{254} TLC plate (E.merck) as absorbent and developed the plate using solvent systems, toluene : ethyl acetate 9:1 and 1: 1 respectively. After developing, the plates were dried and observed the colour spots at UV-254, UV-366 nm and vanillin-sulphuric acid spraying reagent (Wagner *et. al*., 1984).

2.5. WHO parameters

The microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

2.6. Antibacterial activity

The in-vitro antibacterial activity of the drug Jawarish-e-Zanjabeel was performed using the Cup plate method (Anonymous, 1996). The required amount of Muller hinton agar plates were prepared and swabbed with three clinical isolates of *E.coli* coded as *JZECO*-1*, JZECO*-2*, JZECO*-3*,* three clinical isolates of *Klebseilla* spp coded as *JZKP-1, JZKP-2, JZKP-3* and three clinical isolates of *Staphylococcus* spp coded as *JZSS-1, JZSS-2, JZSS-3* along with the standard reference culture *E.coli* ATCC 25922 after confirmation using NCBI database. The plates were allowed to stand for few minutes. Approximately 6mm diameter wells were made using the agar gel borer and 100µl of 50mg/ml conc of the drug dissolved in the solvent DMSO was added into the well (Howard C Ansel et al.,1969) . The commercially available drug ampicillin (10mcg/disc) was used as positive control. The plain disc with100µl loaded solvent DMSO was also placed as the vehicle control. The plates were incubated at 37°C for 24 hours.

2.7. Determination of Minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of the drug required to inhibit the microorganism was also determined by the agar diffusion method and by cup plate method (Anonymous 1982). A series of petridishes containing 20ml of Muller hinton agar media incorporated with increasing concentration of the drug 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml were prepared and allowed to solidify. The bacterial isolates were spot inoculated into each plate. The lowest concentration of the drug that completely inhibits the growth was determined after overnight incubation at 37°C

3. Results and Discussion

Jawarish-e-Zanjabeel is brown, semi solid with agreeable odour and sweetish bitter in taste. The drug was spreaded in a petridish and observed, it did not show any filth, fungus or objectionable extraneous matter.

3.1. Physico-chemical analysis

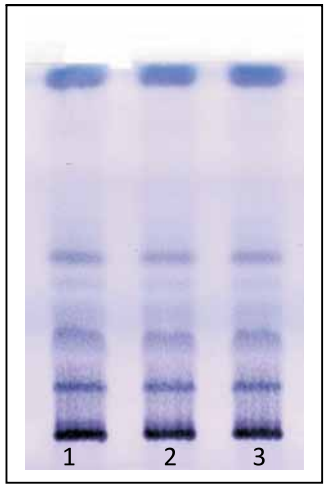
Moisture content of this drug shows 22.08%. The alcohol soluble extractive (38.20%) might be due to the extraction of polar chemicals constituents and the water soluble extractives 58.36% indicate the presence of inorganic constituents. The Physico-chemical data of the drug are shown in Table - I.

Table-1. Analysis of physico-chemical parameters

S. No.	Parameters Analyzed	Batch -I	Batch -II	Batch -III
1	Extractives Alcohol soluble matter (%) Water soluble matter (%)	 38.68 58.72	 37.80 57.84	 38.12 58.52
2	Ash Total ash (%) Acid insoluble ash (%)	 0.82 0.22	 0.91 0.31	 0.79 0.20
3	pH values 1% Aqueous solution 10% Aqueous solution	 5.80 4.61	 5.81 4.74	 5.71 4.63
4	Sugar estimation Reducing sugar (%) Non-reducing sugar (%)	 39.39 9.85	 39.56 9.52	 39.75 10.09
5	Moisture (%)	22.06	22.29	21.89
6	Bulk Density	1.3806	1.4009	1.4208

3.2. Thin Layer Chromatography analysis

Thin layer chromatography studies of chloroform and alcohol extract of all the three batch samples showed identical spots under UV-254,366nm and vanillin-sulphuric acid reagent. The R_f values of the chloroform and alcohol extracts were shown in Table - II and III. The plates were derivatised using vanillin-sulphuric acid reagent and heated at 105º till the color spots appeared (Fig. 1 & 2).



Track 1. Batch - I
Track 2. Batch - II
Track 3. Batch - III

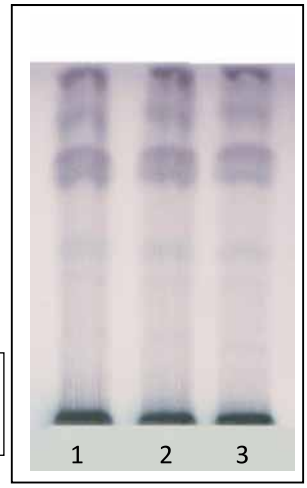


Fig. 1. Chloroform Extract
Solvent system: Toluene: Ethyl acetate (9 : 1)
Detector: V. S. Reagent

Fig. 2. Alcohol Extract
Solvent system: Toluene: Ethyl acetate (1 : 1)
Detector: V. S. Reagent

Table-2: R_f Values of chloroform extract

Solvent system	Rf Values		
Toluene: Ethyl acetate (9 : 1)	UV 254nm	UV 366nm	V. S. Reagent
	0.84 Light pink	0.84 Blue	0.95 Violet
	0.47 Pink	0.54 Light blue	0.81 Light grey
	0.20 Light pink	0.20 Blue	0.47 Grey
	0.15 Pink	0.11 Light blue	0.40 Light grey
	0.11 Light pink		0.31 Light grey
			0.25 Violet
			0.12 Violet

Table-3: R_f Values of alcohol extract

Solvent system	Rf Values		
Toluene: Ethyl acetate (1 : 1)	UV 254nm	UV 366nm	V. S. Reagent
	0.86 Light pink	0.79 Blue	0.95 Violet
	0.79 Pink	0.75 Pale blue	0.84 Pink
	0.68 Light pink	0.20 Violet	0.75 Violet
	0.56 Light pink	0.15 Blue	0.68 Violet
	0.45 Pink		0.47 Grey
	0.30 Light pink		0.37 Light grey
	0.13 Light pink		0.20 Grey

3.3. Heavy metals, microbial load, aflatoxins, pesticide residues

The study carried out on heavy metals such as lead and mercury were present within the permissible limit and other elements cadmium and arsenic were found below the detection limit (Table - IV). The microbial load was found within the permissible limit (Table - V). The studies of other parameters like aflotoxins such as B_1, B_2, G_1 and G_2 were not found in the drug (Table –VI). The pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion were also not detected in the drug samples (Table – VII).

Table-4: Estimation of heavy metal

S. No.	Name of the metal	Results	WHO & FDA Limits
1	Arsenic	Below detection limit	10 ppm
2	Cadmium	Below detection limit	0.30 ppm
3	Lead	0.0216 ppm	10 ppm
4	Mercury	0.0343 ppm	1.0 ppm

Table-5: Analysis of microbial load

S. No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	1,000 CFU / gm	105 CFU / gm
2	Total Fungal Count	Absent	103 CFU / gm
3	Enterobacteriaceae	Absent	103 CFU / gm
4	Salmonella	Absent	Absent
5	Staphylococcus aureus	Absent	Absent

Table-6: Estimation of Aflatoxins

S. No	Aflatoxins	Results
1	B1	Absent
2	B2	Absent
3	G1	Absent
4	G2	Absent

Table-7: Analysis of pesticide residue

Sl. No.	Pesticide residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND
11	Fenthion	ND
12	Heptachlor	ND
13	Lindane	ND
14	Methoxychlor	ND
15	Phorate sulfoxide	ND
16	Phorate sulfone	ND
ND – Not detected		

3.4. Antibacterial activity and MIC

Of the 25 urine samples collected from the patients, 3 isolates were confirmed for *Escherichia coli (JZECO-1, JZECO-2, JZECO-3),* three isolates for *Klebsiella pneumoniae (JZKP-1, JZKP-2 JZKP-3)* and three were identified as *Staphylococcus saprophyticus (JZSS-1, JZSS-2, JZSS-3)* while the remaining were found to be other organisms. The advent discovery of PCR and the sequencing technology enabled the easier and accurate identification of the organism. The drug Jawarish-e-Zanjabeel exhibited higher degree of activity against all the *E.coli* isolates. *Klebsiella pneumoniae* exhibited moderate level of sensitivity whereas the *Staphylococcus saprophyticus* did not show any activity. The zone diameter varies from 20mm to 25mm in case of *E.coli* isolates and between 11mm to 15mm in case of *Klebseilla* isolates at the concentration of 50mg/ml. The MIC study revealed the MIC value as 6.25mg/ml for *Escherichia coli* whereas 1.25mg/ml for the *Klebsiella pneumoniae* (Table-VIII and Fig. 3 & 4).

Table-8: Minimum Inhibitory Concentration

S.No.	Organisms	Concentration of the drug (n = 2)			
		25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
1	JZECO -1	+	+	+	-
2	JZECO-2	+	+	+	-
3	JZECO-3	+	+	+	-
4	JZKP-1	+	+	-	-
5	JZKP-2	+	+	-	-
6	JZKP-3	+	+	-	-
(+) - Presence of activity			(-) - Absence of activity		

Escherichia coli

Disc concentration
1-50 mg/ml
2-25 mg/ml
3-12.5 mg/ml
4-6.25 mg/ml
5-3.125 mg/ml

Klebseilla pneumoniae

Conclusion

The results of present investigation clearly indicate that the drug is free from Microbial growth, Heavy metals, Aflatoxins and Pesticidal residues. The Antibacterial study revealed that the drug Jawarish-e-Zanjabeel is possible source to obtain new and effective herbal medicine to treat infections caused by urinary tract pathogens.

Acknowledgement

The authors are extremely thankful to the Director General, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

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