Development of Pharmaco-poeial Standards and Microbial Studies on Jawarish-e-Qaiser*

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Abstract

Herbal medicines are called as natural products of traditional system of medicines such as Unani, Ayurveda and Siddha. In Unani system of medicine, these herbal products are being prepared using different parts of plants such as root, stem, bark, leaves, flowers and seeds etc. Jawarish-e-Qaiser is one such type of polyherbal Unani formulation prepared by using ten single drugs. The physicians of unani system of medicine have considered this drug as one of the important Unani formulation for the ailments of Qulang (Stomach and Bowel disorder) and Qabz-e-Muzmin (Chronic constipation). The present study was aimed to evaluate pharmacopoeial standards, quality control standards and antimicrobial activity of the drug Jawarish-e-Qaiser. The organisms used in the study were eight type bacterial cultures, namely, *Escherichia coli* (NCIM 2931), *Staphylococcus aureus* (NCIM 5021), *Enterobacter aerogens* (NCIM 5139), *Bacillus subtilis* (NCIM 2197), *Pseudomonas aeruginosa* (NCIM 2945), *Salmonella typhimurium* (NCIM 2501), *Bacillus cereus* (NCIM 2458), *Pseudomonas putida* (NCIM 2847) and one fungal (yeast) culture *Candida albicans* (NCIM 3471). The study revealed that the obtained data of quality control parameters were within the permissible limits of WHO and the drug shows potent antimicrobial activity against all the tested organisms at 100mg/ml concentration. The MIC was found to be within the range of 1.562µg/µl to 3.125µg/µl for majority of the organisms tested. Comparatively the drug was found to be least effective against both the tested *Pseudomonas* spp.

Keywords: Jawarish-e-Qaiser, Physico-chemical, Quality control, Antimicrobial activity.

Introduction

In modern era the phytomedicines have become potential medicines to cure variety of diseases. The usage of traditional medicines such as Unani, Ayurveda and Siddha have increased in both developing and developed countries due to their natural origin and lesser side effects (Mukerjee, 2008). As the world's population relies on herbal based medicines, search for novel antibacterial and antifungal agents' validation of scientific standards has becomes necessary issue to establish safe and efficacious drugs. In this context, quality standardization and biological activities of Unani medicine

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will open new frontiers for treatment of several ailments (Sharma and Arora, 2006). The drug Jawarish-e-Qaiser is one of the polyherbal Unani formulation listed in the National Formulary of Unani Medicine (NFUM, Part - IV). Literature studies revealed that some ingredients of the formulation have been reported to possess antimicrobial activity (Kaushik, 2011; Arora and Kaur, 2007)

Hence the present study was aimed to evaluate the pharmacopoeial and antimicrobial activity of Jawarish-e-Qaiser by using scientific methods.

Materials and Methods

Collection of raw drugs and preparation

To develop scientific method for the preparation of drug, raw drugs were procured from local raw drug dealers, Chennai. All the raw drugs were identified and authenticated using pharmacognostical methods (Kokate *et al*., 2000). Jawarish-e-Qaiser was prepared in three different batches using ten raw drugs namely, Tukhm-e-Karafs (*Apium graveolens* Linn. DSM - 81), Nankhwah (*Trachyspermum ammi* (L) Sprague ex. Turril. DSM - 83), Aaqarqarha (*Anacyclus pyrethrum* DC. DSM - 8), Namak Lahori (Rock salt), Filfil Daraz (*Piper longum* Linn. DSM - 45), Zanjabeel Khushk (*Zingiber officinale* Rosc. DSM - 86), Halela Zard (*Terminalia chebula* Retz. DSM - 64), Saqmonia (*Convolvulus scammonia* Linn. DSM - 148), Turbud Safaid (*Operculina turpethum* Linn. DSM - 151) and Qand Safaid (Sugar) as per the guidelines of NFUM Part-IV (Anonymous, 2006).

Collection of microorganism

To evaluate the microbial studies, the typed cultures were procured from National Chemical laboratory (NCL) Pune. The organisms used were *Escherichia coli* (NCIM 2931), *Staphylococcus aureus* (NCIM 5021), *Enterobacter aerogens* (NCIM 5139), *Bacillus subtilis* (NCIM 2197), *Pseudomonas aeruginosa* (NCIM 2945), *Salmonella typhimurium* (NCIM 2501), *Bacillus cereus* (NCIM 2458), *Pseudomonas putida* (NCIM 2847) and *Candida albicans* (NCIM 3471). All the organisms were confirmed using specific biochemical tests (Mackie & McCartney, 1996).

Physicochemical analysis

All the three batch samples of the drug were subjected to evaluate physico-chemical studies (Anonymous, 1987).

Thin Layer Chromatographic Studies

TLC studies of chloroform and alcohol extracts of the drug samples were performed using the standard methods (Wagner *et al*., 1984).

Quality Control parameters

To evaluate quality of the drug samples, parameters viz., microbial content, heavy metals, aflatoxin and pesticide residues were studied using WHO guidelines (Anonymous, 1998; 2000).

Microbial studies

Inoculum Preparation

A uniform suspension of the organisms listed above were prepared in 6ml of saline, and compared with the McFarland's standards (Mackie & McCartney, 1996). Each microbial suspension was diluted with the saline to a density visually equivalent to the Barium sulphate standard, 0.5 McFarland's unit. The plates were inoculated within 15 minutes of the preparation of the suspension to avoid changes in the density of the cultures.

Preparation of plates and Inoculation of microbial cultures

The required quantities of the Muller Hinton agar were prepared. The pH of medium was adjusted to 7.2. Each plate was poured with 20ml of the media and was allowed to solidify. The tubes containing 0.5 McFarland's unit equivalent microbial cultures were dipped with sterile cotton swabs, and excess of the fluid was removed by gently rotating the swabs against the sides of the test tube. The dipped swabs were swabbed over the Muller Hinton agar plates covering the entire surface of the plate by rotating the plates in all the directions. After solidification wells of 6 mm diameter were punched in agar plates. Plates were then allowed to set for few minutes.

Drug concentration

1gm of drug Jawarish-e-Qaiser was accurately weighed and dissolved in 10ml of DMSO solvent (Divakar and Nair, 2001) to make the stock solution containing 100mg/ml concentration. A series of dilutions were made from the stock solution to obtain 100µg/µl, 50µg/µl, 25µg/µl, 12.5µg/µl, 6.25µg/µl, 3.125µg/µl, 1.5625µg/µl, and 0.78µg/µl for determination of MIC.

Antibacterial assay and Determination of Minimum Inhibitory Concentration (MIC)

Antibacterial activity was assayed in duplicates by agar well diffusion method (Vasudha Rai, *et al*., 2011) using the above mentioned test organisms. The well was loaded with 50µl of the drug (100mg/ml conc.). The commercially available drug Norfloxacin (10mcg/disc) was used as control. The plain disc with 50µl loaded solvent DMSO was placed as the vehicle control. The plates were incubated at 37°C for 24 hours. The diameter of the clearing zones were measured in mm using the calipers.

The precise assessment of the effectiveness of the drug Jawarish-e-Qaiser against the susceptible bacteria was achieved by determining the MIC with varying concentration ranging from 0.78µg/µl to 100 µg/µl by agar well diffusion method. The plates were incubated at 37□C for 24hrs and were observed for the MIC, which was read as the lowest concentration of the drug required to completely inhibit the growth of the organism.

Results and Discussion

Physico-chemical analysis

The evaluated physico-chemical data of the drug are shown (Table – 1).

Thin Layer Chromatographic Studies

The TLC studies of the chloroform and alcohol extracts of all the three batch samples showed identical spots under UV - 254nm, 366nm and VS reagent. The Rf values of the chloroform and alcohol extracts are shown (Table 2 & 3, Fig. 1 & 2.)

Quality Control parameters

The study carried out on analysis of heavy metals, microbial load, aflatoxins and pesticide residues were shown (Table 4, 5 6 & 7) respectively.

Antimicrobial activity study and MIC

Antibacterial activity was studied against eight bacterial cultures viz., *Escherichia coli* (NCIM 2931), *Staphylococcus aureus* (NCIM 5021), *Enterobacter aerogens* (NCIM 5139), *Bacillus subtilis* (NCIM 2197),

Pseudomonas aeruginosa (NCIM 2945), *Salmonella typhimurium* (NCIM 2501), *Bacillus cereus* (NCIM 2458), *Pseudomonas putida* (NCIM 2847) and one yeast culture *Candida albicans* (NCIM 3471). A significant growth inhibition was shown by most of the organisms tested indicating the profound potency of the drug Jawarish-e-Qaiser. Among the tested organism *Salmonella typhimurium* was found to be the most sensitive organism followed by *Candida albicans, Escherichia coli, Bacillus spp., Staphylococcus aureus* and *Enterobacter aerogens* with zone of diameter ranging from 26mm to 7 mm (MIC Conc 100µg/µl to 12.5µg/µl). Both the species of *Pseudomonas* organism exhibited only minimum sensitivity to the drug with zone of diameter ranging from 11 mm to 7 mm (MIC Conc 100µg/µl to 12.5µg/µl). The results of the antibacterial activity and MIC of the drug Jawarish-e-Qaiser for all the organisms were observed and tabulated (Table 8, Fig. 3).

Table 1 : (Physico-chemical parameters)

Parameters Analyzed	Batch Number (n = 3)		
	I	II	III
Extractives Alcohol soluble matter Water soluble matter	48.84% 64.52%	49.04% 64.80%	48.52% 65.04%
Ash Total ash Acid insoluble ash	 2.13% 0.061%	 2.32% 0.055%	 2.46% 0.048%
pH values 1% Aqueous solution 10% Aqueous solution	 5.53 4.34	 5.79 4.52	 5.49 4.46
Sugar estimation Reducing sugar Non-reducing sugar	 38.39% 9.15%	 38.43% 9.24%	 38.41% 9.19%
Moisture	19.30%	19.84%	19.46%
Bulk Density	1.4099	1.4265	1.4205

Table 2 : (R_f values of Chloroform extract)

Solvent System (Toluene: Ethylacetate) (9:1) (Fig. 1)	Rf values		
	UV 254 nm	UV 366 nm	V.S. Reagent
	0.93 Grey 0.78 Blue 0.65 Pink 0.56 Pink 0.46 Pink 0.37 Light Pink 0.30 Light Pink 0.24 Pink 0.12 Pink	0.93 Yellowish Brown 0.78 Blue 0.72 Blue 0.56 Blue 0.46 Blue 0.41 Light Blue 0.34 Blue 0.29 Light Blue 0.21 Blue	0.93 Yellowish Green 0.80 Grey 0.72 Light Grey 0.56 Violet 0.46 Light Grey 0.26 Grey 0.12 Grey

Table 3 : (R_f values of alcohol extract)

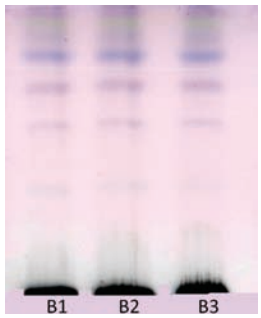
Solvent System (Toluene:Ethylacetate) (6:4) (Fig. 2)	Rf values		
	UV 254 nm	UV 366 nm	V.S. Reagent
	0.93 Pink 0.85 Light Pink 0.68 Pink 0.52 Pink 0.31 Light Pink	0.93 Yellow 0.85 Fluorescent Blue 0.80 Blue 0.73 Blue 0.65 Yellowish Green 0.56 Blue	0.93 Yellowish Green 0.86 Grey 0.81 Blue 0.71 Grey 0.58 Grey 0.36 Light Grey 0.20 Light Grey

Table 4 : Estimation of Heavy Metals

Parameters	Results	WHO/API Limits
Lead	0.0351ppm	10 ppm
Cadmium	Nil	0.3 ppm
Mercury	Nil	1 ppm
Arsenic	Nil	3 ppm

Table 5 : Estimation of Microbial load

Parameters	Results	WHO Limits for internal use
Total Bacterial Count (TBC)	$8x10^1$cfu/g	$1x10^5$cfu/g
Total Fungal Count (TFC)	$1x10^2$ cfu/g	$1x10^3$cfu/g
Enterobacteriaceae	Absent	$1x10^3$cfu/g
Escherichia coli	Absent	$1x10^1$cfu/g
Salmonella spp	Absent	Absent
Staphylococcus aureus	Absent	Absent

Table 6 : Estimation of Aflatoxin

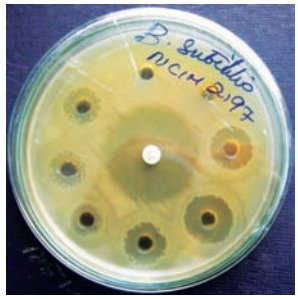
Aflatoxin	Results	Detection limit
B1	Nil	DL:1.0 ppb
B2	Nil	DL:0.5 ppb
G1	Nil	DL:1.0 ppb
G2	Nil	DL:0.5 ppb

Table 7 : Estimation of Pesticidal residue

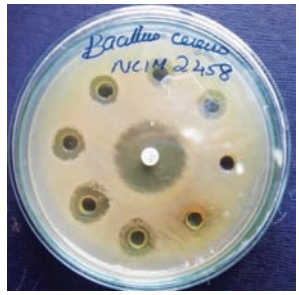
S. No.	Pesticide residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND
11	Fenthion	ND
12	Heptachlor	ND
13	Lindane	ND
14	Methoxychlor	ND
15	Phorate sulfoxide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

Table 8. Antimicrobial activity (MIC)

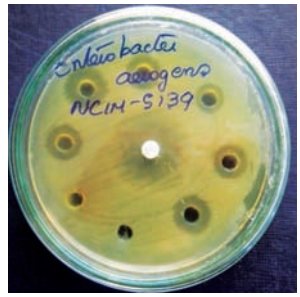
Sl. No.	Organisms	Zone diameter in mm								
		100 µg/µl	50 µg/ µl	25 µg/ µl	12.5 µg/ µl	6.25 µg/ µl	3.125 µg/ µl	1.562 µg/µ1	0.781 µg/µ1	Std (Nor)
1	*Escherichia coli* (NCIM 2931)	24	22	20	17	15	14	-	-	S
2	*Staphylococcus aureus* (NCIM 5021)	18	17	16	14	12	9	8	7	S
3	*Enterobacter aerogens* (NCIM 5139)	18	17	15	14	13	10	8	-	S
4	*Bacillus subtilis* (NCIM 2197)	22	19	18	13	12	10	9	-	S
5	*Pseudomonas aeruginosa* (NCIM 2945)	10	9	8	-	-	-	-	-	S
6	*Salmonella typhimurium* (NCIM 2501)	26	25	24	23	22	15	13	-	S
7	*Bacillus cereus* (NCIM 2458)	19	15	14	12	11	10	-	-	S
8	*Pseudomonas putida* (NCIM 2847)	11	10	8	7	-	-	-	-	S
9	*Candida albicans* (NCIM 3471)	25	24	23	22	19	16	13	-	S
Nor: Norfloxacin; S: Sensitive										



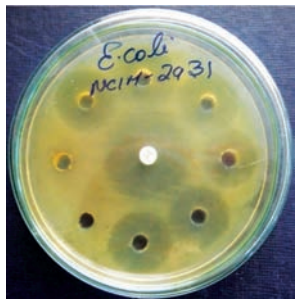
Bacillus subtilis
NCIM 2197



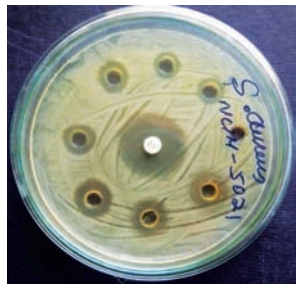
Bacillus cereus
NCIM 2458



Enterobacter aerogens
NCIM 5139

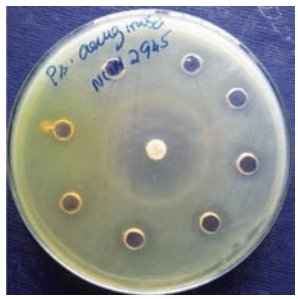


Escherichi coli
NCIM 2931

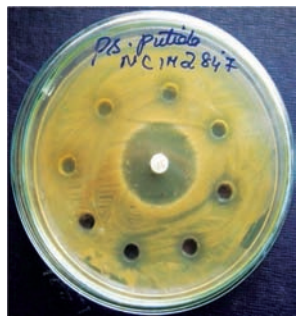


Staphylococcus aureus
NCIM 5021

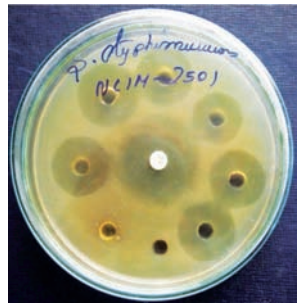
1. 100µg/µl
2. 50 µg/µl
3. 25 µg/µl
4. 12.5 µg/µl
5. 6.25 µg/µl
6. 3.125 µg/µl
7. 0.78 µg/µl
8. Vehicle control
9. Std (Norfloxacin)



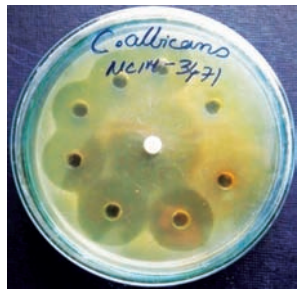
Pseudomonas aeruginosa
NCIM 2945



Pseudomonas putida
NCIM 2847



Salmonella typhimurium
NCIM 2501



Candida albicans NCIM 3471

Fig. 3: Plates showing antimicrobial activity of Jawarish-e-Qaiser

Conclusion

The results of the present investigation on physicochemical parameters and quality control parameters clearly emphasizes that the drug Jawarish-e-Qaiser is free from toxic substances indicating the safety and purity of the drug. Antimicrobial activity indicates that the drug possesses the antimicrobial property and it can be used as an alternative medicine.

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