



## Original Article

# Microbial Analysis of *Majoone Falasifa* (A Compound Herbal Formulation)

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### ABSTRACT

*Majoone Falasifa (MF)* is a polyherbal sugar based semisolid preparation used in Unani medicine to treat neurological, digestive, urinary and various chronic and debilitating disorders especially in geriatric care. This Unani compound formulation contains thirteen ingredients viz; *Emlica Officinalis*, *Matricaria Chamomillia*, *Cinnamomum Zeylanicum*, *Piper Longum*, *Piper Nigrum*, *Pinus Gerardiana*, *Cocus Nucifera*, *Vitis Vinifera*, *Terminalia Bellirica*, *Orchis Latifolia*, *Plumbago Zeylanica*, *Zingiber Officinalis* and *Aristolochia Indica*. Though used since ancient time its microbial analysis was not carried out till date. Therefore in present study microbial analysis of *Majoone Falasifa* was evaluated under the accelerated testing conditions.

**Key words:** *Majoone Falasifa*, Microbial analysis, Unani Medicine

### INTRODUCTION

*Majoon* is a semisolid medicinal preparation where one or more single drugs of plant, animal or mineral origin are mixed in powder or liquid form in base (*qiwwam*) made of purified sugar, honey, candy or jaggary <sup>1</sup>. *Majoone Falasifa* is a wonder drug belonging to Unani system of medicine. It is a compound formulation consisting of fourteen ingredients with sugar/honey as base as mentioned in National Formulary of Unani Medicine Part -1<sup>1</sup>. Although there is minor variation in number and weight of ingredients in different Unani compilations, such is its reputation that it has been coined as *Madaat-ul-Hayat* i.e. elixir of life <sup>2</sup>. *Hakim Akbar Arzani* in his compilation *Qarabadeen Qadri* has mentioned that this *Majoon* was compounded by *Indroomakhas* of Greece in consultation with other physicians of his time and it was named *Majoone Falasifa* by Arab physicians and is being prescribed for past two thousand years with promising results <sup>3</sup>. The spectrum of therapeutic activity of *Majoone Falasifa* is wide. If the formulations belonging to Unani system of medicine are rowed according to their reputation and results, *Majoone Falasifa* will surely find place in first row because of its prompt therapeutic action, not only this, if we talk of scope of medicinal activity of *Majoone Falasifa*, we reach to the conclusion that its therapeutic activity is not restricted to the disorders of one system or organ. It can be used with confidence in disorders of almost all systems and organ of human body. The disorders related to nervous system like weakness of nerves, memory weakness can be best treated with this *Majoon* because it strengthens the nerves, enhances capacity to memorize and boosts the intellect. The disorders related to digestive system like loss of appetite, indigestion and flatulence can be managed by this *Majoon* because of its appetizer, digestive and anti-flatulent activity. The disorders related to bones like arthritis, lumbago and weakness of bones and joints if treated with this *Majoon* gives promising results. The therapeutic activity of *Majoone Falasifa* is equally applicable in disorders like sexual debility, urinary incontinence, halitosis, weakness of teeth and gingivitis etc <sup>2,4,5</sup>.

### METHODOLOGY

In the present study microbial analysis was done for total bacterial count, total fungal count and presence of specific pathogenic viz; *E.coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* in the samples of test drug formulation. The following tests are designed for the estimation of the number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in

*Majoone Falasifa*. The term 'growth' is used to designate the presence and presumed proliferation of viable micro-organisms.

**Pretreatment of sample:** Ten g of *Majoone Falasifa* was dissolved in buffered sodium chloride-peptone solution pH 7.0 and volume was adjusted to 100 ml with the same medium. In case of sample to be examined for the specific pathogenic count lactose broth was used in place of buffered sodium chloride-peptone solution.

**Procedure for total bacterial/fungal count:** Membrane filtration method was used for total bacterial/fungal count. Membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm were used.

Ten ml of each dilution containing 1 g of the preparation being examined were transferred to each of two membrane filters and filtered immediately. Each membrane was washed by filtering through it three or more successive quantities, each of about 100 ml, of buffered sodium chloride-peptone solution pH 7.0. One of the membrane filters, intended for the enumeration of bacteria, was transferred to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, was transferred to the surface of a plate of Sabouraud dextrose agar with antibiotics. Plates were incubated for 5 days, at 30°C in the test for bacteria and 20°C in the test for fungi. Number of colonies that formed was counted and number of micro-organisms was calculated per g of the preparation being examined.

#### Tests for specific pathogens:

***Escherichia coli*:** One gram of sample was placed in a sterile screw-capped container, 50 ml of nutrient broth was added, shaken, allowed to stand for 1 hour and shaken again. The cap was loosen and incubated at 37°C for 18 to 24 hours.

**Primary test:** One ml of the enrichment culture was added to a tube containing 5 ml of MacConkey broth and incubated in a water-bath at 36°C for 48 hours. There was no formation of acid and gas in the tube thus there was no need to carry out the secondary test and absence of *Escherichia coli* was thereby confirmed.

***Salmonella*:** The pre-treated preparation of *Majoone Falasifa* containing one g of the product was transferred to 100 ml of nutrient broth in a sterile screw-capped jar, shaken, allowed to stand for 4 hours and shaken again. The cap was loosened and incubated at 35°C for 24 hours. 1.0 ml of the enrichment culture was added to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubated at 36°C for 48 hours. From each of these two cultures subculture was carried out on *bismuth sulphate agar* and *brilliant green agar media*. The plates were incubated at 36°C for 20 hours. Upon examination, if the selected media does not meet the description of colony (colour change) then there is absence of *salmonella* in the sample under investigation.

***Pseudomonas aeruginosa*:** Hundred ml of fluid soyabean-casein digest medium was inoculated with 1 g of pre-treated sample preparation being examined. It was mixed and incubated at 35°C for 30 hours. Medium was examined for growth. No growth in the medium confirms the absence of *Pseudomonas aeruginosa* in the sample of *Majoone Falasifa*.

***Staphylococcus aureus*:** Same process as carried in case of *Pseudomonas aeruginosa* was followed and instead of soyabean-casein digest medium, *Vogel-Johnson agar* medium was used. Upon examination of the incubated plates, none of them contained colonies having the characteristics colonial morphology i.e. black spot surrounded by yellow zones, thus confirming the test is negative for "*staphylococcus aureus*"<sup>6</sup>.

## RESULTS AND DISCUSSION

Microbial analysis was done by evaluation of total bacterial, total fungal and specific pathogenic count in test drug samples at 0,1,3 and 6 months. Total bacterial count in *Majoone Falasifa* (MF) at accelerated conditions at zero, one, third and six month was 305, 250, 3300 and 2450 Cfu/gm/ml as shown in the table no 1.

**Table 1. Total bacterial and fungal count of *Majoone Falasifa* in ASS (Accelerated stability samples)**

Sample	Total bacterial count (Cfu/gm/ml)	WHO Limit (Cfu/gm/ml)	Total fungal count (Cfu/gm/ml)	WHO Limit (Cfu/gm/ml)	Inference
0 Month	305	10 <sup>5</sup> /gm	10	10 <sup>3</sup> /gm	Within limit
1 Month	250	10 <sup>5</sup> /gm	10	10 <sup>3</sup> /gm	Within limit
3 Month	3300	10 <sup>5</sup> /gm	10	10 <sup>3</sup> /gm	Within limit
6 Month	2450	10 <sup>5</sup> /gm	10	10 <sup>3</sup> /gm	Within limit

Total fungal count of *Majoone Falasifa* at all study time point were 10Cfu/gm/ml as shown in the table no 1.

Specific pathogenic count was absent in all samples as shown in Table no 2.

**Table 2: Presence of pathogenic bacteria in ASS (Accelerated stability samples) of Majoone Falasifa**

Sample	<i>E. coli</i>	<i>Salmonella</i>	<i>Staph. aureus</i>	<i>P. aeruginosa</i>
0 Month	Absent	Absent	Absent	Absent
1 Month	Absent	Absent	Absent	Absent
3 Month	Absent	Absent	Absent	Absent
6 Month	Absent	Absent	Absent	Absent

These values of bacterial and fungal estimation fall under the acceptance limit i.e.  $10^5$ /gm for total bacterial count and  $10^3$ /gm for total fungal count according to WHO <sup>7</sup>. As the microbial test of *Majoone Falasifa* displayed accepted level of microbial load, it can be concluded that the test drug formulation is microbiologically stable at accelerated storage conditions.

The probable factors as to why the test drug formulation was able to retain microbial stability seems to be excellent *qiwam* (consistency) quality which dehydrated the microbes by creating osmotic pressure and impede growth of microbes <sup>8</sup>. Besides, the test drug formulation was immediately packed in high quality air tight containers which would have restricted the entry of moisture and air in to the drug and prevent hydrolysis and oxidation. The other important factor behind the stability of test formulation might be cleaning and washing of the ingredients of test drug formulation by distilled water and subsequent drying thereafter at 60°C, as both these procedures have proven ability to limit the microbial load of substances <sup>9</sup>. *Qiwam* of *Majoone Falasifa* was also pre prepared in distilled water. Further results of low bacterial load in the samples of the test drug can be attributed to the low acidic pH of the test drug formulation, as it has been observed that herbal preparations show lower limits of microbial status in case of low acidic pH <sup>10</sup>.

## CONCLUSION

Above discussion describes clearly that the results in case of total microbial counts were under accepted limits as per WHO guideline. Specific counts were also negative in all the samples of the test drug formulation. Therefore, it is obvious from the above statement that test drug formulation retained microbial stability under accelerated testing conditions of storage.

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CONFLICT OF INTEREST : Nil  
 Received : 01.06.2015  
 Accepted : 10.08.2015

## CITATION OF THIS ARTICLE

Peerzada Mohd. Y, Khaleequr R, Basharat R, Seema R. Microbial Analysis of *Majoone Falasifa* (A Compound Herbal Formulation). *World J. Clin. Pharmacol. Microbiol. Toxicol.* Vol 1 [3] September 2015. 32-34