Original article:

Anthelmintic activity of *Majoon Sarakhs* on Earthworm

Ghausia Islam[1], Nasreen Jahan[2]

Abstract:

Objective: Toevaluate the anthelmintic activity of *Majoon Sarakhs*, a pharmacopoeal Unani formulationonadult earthworm(*Eiseniafetida*). ***Methods:*** Adult earthworms of similar age, size and weight were divided into six groups of 6 each and released in petridishes containing 0.5% CMC in normal saline (control), Albendazole (standard), hydro alcoholic extract (HAEMS), aqueous extract (AEMS), *Majoon* (MS) and powder (PFMS) of ingredients of *MajoonSarakhs* in various concentrations (50, 100, 150, 200, 250 and 300 mg/ml). Time for paralysis was noted when no movement was observed. Death was confirmed when the worms neither moved when shaken vigorously nor when dipped in warm water followed by fading away the body colours. Phytochemical analysis and HPLC finger printing were also carried out. ***Results:*** All dosage forms of ingredients of MS showed significant anthelmintic ($p<0.001$) effect in concentration dependent manner when compared to Albendazole. The test formulation paralysed and killed the worms at all concentration, but at higher concentration it took shorter time. The order of activity was HAEMS > AEMS > Albendazole > MS > PFMS at 50mg/ml.At higher dose MS showed better results than Albendazole. ***Conclusion:*** on the basis of above results it can be concluded that MS and extracts of its ingredients possess profound anthelmintic activity against tested worms. It validates the claims of Unani physicians that MS is a good anthelmintic agent.

Keywords: Albendazole; Anthelmintic; Earthworms; Intestinal helminthiasis; *MajoonSarakhs*.

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Abbreviations: CMC: Carboxy Methylcellulose, *Majoon Sarakhs*: MS, NS: Normal Saline.

1. Introduction

Helminth infections are among the commonest form of parasitic infections in man, affecting a large proportion of the world's population, especially in children. In developing countries they pose a major risk to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. The World Health Organization estimates that over two billion people are afflicted with helminthiasis[1]. It is approximated that by the year 2025, about 57% of the population in developing countries will be predisposed[2]. Helminth infections are now being acknowledged as a root of many acute and chronic health condition among the human beings as well as the cattle. More than half of the population of the world suffers from infection of one or the other and majority of cattle suffers from worm infections[3]. In most developing and under developed countries, helminthicinfections are a leading health concern because they predisposehumans to other infections such as fungal and bacterial [4].

All the Unani physicians are of the common opinion that excessive putrefied phlegmis the sole cause of production of intestinal helminthiasis. About 197 species of helminths have been found in association with the human alimentary tract[5]. Roundworms, hookworms, and whipworms thrive in human communities in which poverty is entrenched andwhere there arelack of clean drinking water; poor sanitation, health care, and health awareness[6]. Worms are

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grouped into three categories: Nematoda, Cestoda and Trematoda. For these three categories, three groups of drugs are used viz. Antinematodal, Anticestodal and Antitrematodal drugs. All anthelmintic basically kill worms by either starving them to death or paralyzing them since worms have no resources of storing energy, they must eat incessantly to meet their metabolic requirements. Any interruption in this process results in energy depletion. Interfering with feeding for 24 hours or less is sufficient to kill most adult helminths. Helminths will also die if they become paralyzed and temporarily lose their capability to uphold their position in the gut[7]. There are some potential chemotherapeutic targets which include energy metabolism, nutrient uptake, nucleic acid metabolism and anabolic pathways[8]. Rational control of helminthic infections involves the regular use of appropriate anthelmintic drugs. However, continuous administration of a drug leads to the development of resistance[9]. Moreover; most of the existing anthelmintic drugs produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea.[10] In traditional medicine, at least 80% of the world's population in developing countries uses plant material as their source of primary health care[11]. Rising problems of development of resistance in helminths against anthelmintics and the impact of conventional anthelmintics on the environment, it is imperative to look for alternative strategies against gastrointestinal nematodes[12].

Following the folk claims, several medicinal plants have been scrutinized for this activity using various *in vitro*[13,14] and *in vivo* methods[13] such as aqueous extract of *Hing* (*Ferula foetida*) which showed significant anthelmintic activity against earthworm,[15] the ethanolic extract of drupe of *Melia azedarach* reported effect on *Phretima posthma*[14]. The aqueous extract of *Carica papaya* showed significant effect against *Ascaris lumbricoides* and *Ascardia galli*, likewise *Nigella sativa, Piper longum, Commiphora mukul, Trychyspermum ammi* also testified significant anthelmintic effect[14]. Thus, search for more alternative therapeutic agents for the treatment and control of helminthic infections has become essential. Various single and compound drugs have been described in Unani literature to be effective in the treatment of different types of worm infestation and Unani physicians are using them since ages successfully. It has been observed in routine practice that in most of the cases the compound formulations are more effective than the single drug thus for the present study, a Pharmacopoeal Unani formulation *Majoon Sarakhs* (MS) was selected to study its effect on earthworm. MS is a polyherbal formulation rationally designed by Unani physicians containing four ingredients viz. *Sarakhs* (*Dryopteris filix mas*), *Baobarang* (*Embeliaribes*), *Turbud*(*Ipomoea turpethum*),and *Muqil* (*Commiphora mukul*) in different proportion[16]. All ingredients in this formulation are hotanddry in temperament and bitter in taste[17].

Phytoconstituents play a major role in the anthelmintic activity; they may act jointly or separately by inhibition of tubulin polymerization and block glucose uptake[18]. *Dryopteris filix mas* contains vermicidal principle phloroglucinols, such as aspidin, deaspidin, and filixicacid[19]. These are active against intestinal cestodes and probably paralyze the worm's muscles[8].While, *Embeliaribes* has the active constituent embelin, the aqueous and ethanolicextract exhibited anthelminthic effect against earthworms[20]. *Ipomoea turpethum* contains a glycosidic resin, which has the insoluble glycoside turpethein. This purges out the thick and viscous humour[21]. *Commiphora mukul* is a complex mixture of steroids, diterpenoids, aliphatic esters, carbohydrates, amino acids and triglycerides. It has been used as an anti-inflammatory, antispasmodic, anthelmintic etc[22]. The alcoholic extract and essential oil of *Commiphora mukul* has shown good anthelmintic activity against hook worm and tapeworm[23]. *Embeliaribes*[13,20,24] and*Commiphoramukul*[14,22,23] and Dryopteris *filix mas*[13,19] have already been studied for its anthelmintic activity. But *Majoon Sarakhs* as a whole has not been investigated so far. Therefore, in the present study *Majoon Sarakhs* is selected to evaluate the anthelmintic activity on earthworm.

The aim of the present study is to evaluate the effect of ingredient of *Majoon Sarakhs* in different dosage forms. Hence powder, aqueous and hydro alcoholic extract of the ingredient and *Majoon* itself was investigated at different concentration to determine the comparative lethality of all dosage forms in concentration dependent manner. Albendazole was used as standard drug[25] to make the study comparative in the same concentration.

2. Materials and methods

1.1. *Collection of drug materials*

The crude drugs were procured from local market of Bengaluru; identified and authenticated by Dr. S. Noorunnisa Begum, Associate Professor, Centre for Repository of Medicinal Resources (C-RMR) Trans Disciplinary University (TDU) 74/2,

Jarakabande Kaval, and Post: Attur, Via Yelahanka, Bengaluru-560064. Raw drug samples were allotted as FRLHT No. 4575 for *Turbud* [(*Operculina turpethum* (L.) Silva Manso.)], 4576 for *Sarakhs* [(*Dryopteris filix mas* (L) Schott.)], 4577 for *Muqil* [*Commiphora mukul* (Hook. Ex Stocks) Engl)] and 4572 for *Baobarang* [(*Embeliatsjeriam-cottam* (Roem.&Schult.) A. DC.]. The specimens of each drug have been deposited to the Drug Museum / Herbarium, NIUM, Bengaluru with Voucher No. 54/ IA/Res/2018 for record.

2.2. Preparation of extract of ingredient of Majoon Sarakhs

All the ingredients of MSwere powdered by an electric grinder and sieved by Sieve no. 80 to get fine powder. The 50% hydro alcoholic extract (water: ethanol, 50:50 v/v) of the test drugs were obtained by using Soxhlet's apparatus in different drug and solvent ratios i.e. 1:5, 1:10 and 1:15. The extraction was done for 8 h at temperature 80ºC and filtered to obtain a clear filtrate then it was dried on water bath to obtain a solid residue of extract. The maximum yield percentage of aqueous extract was found to be 22.26% with 1:15 drug and solvent ratio while hydro alcoholic extract yielded 24.77% in 1:10 drug and solvent ratio. Further, standardization of the extract was carried out by the qualitative phytochemical analysis. Extracts of above ratio showed the maximum presence of phytoconstituents that is why; the same extracts were used for anthelmintic study. The extracts were stored in air tight container in refrigerator for further experiment.

1.3. *Preparation of Majoon Sarakhs*

All the ingredients of MSwere first washed with water to remove dirt and debris and then subjected to drying at 60ºC in an oven for 4 hours. The drugs were finely powdered by an electric grinder (Sieve no. 80) and stored. *Muqil* were soaked in water and allowed to stand for 24 hrs so that all the gum dissolved in water, then squeezed from a muslin cloth and filtrate was dried in an oven at a temperature less than 50˚C, collected, and the dried powder was passed through a sieve no. 80 and stored carefully in a desiccator until required. All the ingredients were mixed together in appropriate ratio (drug and honey 1:3). Honey heated on fire separately in a vessel. After then, the powdered drugs were mixed thoroughly and stirred till all the ingredients were completely homogenized following that, vessel was removed from the fire and allowed to cool. No preservative was added in the formulation.

1.4. Experimental animal

Most of the screening on anthelmintics reported are *in vitro* studies using Indian earthworm such as *Phretimaposthuma, Eisenia fetida, Ascardia galli, Ascaris lumbricoides*etc.[14] It has been demonstrated that all anthelmintics which are toxic to earthworms are creditable to study as an anthelmintic. They have anatomical and physiological resemblance with the intestinal roundworm parasite found in human beings[26,27]. Because of its easy availability; earthworms have been used extensively for the preliminary *in vitro* evaluation of anthelmintic compounds[28,29]. Therefore, in the present study earthworm is taken as an experimental model to evaluate the anthelmintic activity of *Majoon Sarakhs.* Adult earthworms, *Eisenia fetida* were obtained from the moist soil of Gandhi Krishi Vignana Kendra, (GKVK), Bengaluru, Karnataka. They were kept in the required media at room temperature and maintained their environmental condition throughout the study.

1.5. *Chemicals, solvents and reagents*

All the chemicals and reagents used in this study were of analytical grade and procured from authentic sources. Ethanol was obtained from Changshu Hongsheng Fine chemical Co. Ltd, Jiangsu province. The chemicals used in phytochemical analysis were obtained from Central Drug House (P) Ltd. New Delhi, India. While Tincture alkane was taken from LEO CHEM, Bengaluru, India. Filter paper (Whatman no.40) and Millipore filter paper purchased from GE Healthcare UK Limited, UK. Albendazole, (Zental) of Glaxo Smith Kline Pharmaceuticals Ltd. Bengaluru procured from local medical store of Bengaluru, India.

1.6. *Experimental design*

Indian adult earthworms (*Eisenia fetida*) of 3-5cm in length, 0.1-0.2cm in width and 0.7-1.2 g in weight were used for the study. They were divided into Plain control **(**Normal saline), Test group A **[**Hydro alcoholic extract of the ingredients of MS (HAEMS)], Test group B **[**Aqueous extract of the ingredients of MS(AEMS), Test group C (MS),Test group D [Powder of the ingredients of MS (PFMS)], Standard group **[**Albendazole] containing 6 earthworms in each group. Since the aim of study was to determine the concentration dependent effect of each dosage form therefore, each group of particular dosage form was further subdivided in 6 groups containing 6 earthworms

in each. Before the experiment, the earthworms were washed with normal saline to remove all faecal matter and waste surrounding their body.

1.7. Evaluation of *in vitro* anthelmintic activity

The method of Ramaiah et al, (2013)[25] and Salhan et al., (2011)[30] was followed with some modification in the dose regime.Six earthworms were released into same size of Petri dishes containing normal saline, with varying concentration of each dosage forms such as *Majoon*, powder, aqueous and hydro alcoholic extracts of MSand standard drug Albendazole. The suspension of *Majoon*, powder, hydro alcoholic and aqueous extract of the ingredients of MSat different concentration (50, 100, 150, 200, 250, 300 mg/ml) were prepared by using 0.5% (w/v) of CMC as a suspending agent and final volume was made up to 10 ml for respective concentration by addition of normal saline. Test drug and the standard drug solution of respective concentration were freshly prepared before starting the experiments and poured in different petridishes. While plain control was treated only with 10 ml normal saline. Soon after releasing the earthworm in respective petridishes, they were observed for their spontaneous motility and evoked responses. (Fig. 1-2)

Observations were made for the time taken to paralysis and death of individual worms continuously for 3 h. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death time was noted when the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) even after performing the prick test, (pricking the body of earthworm by a sharp needle followed by pouring of warm water drop by drop on them) followed by fading away of their body colours. For this purpose the worms along with the Petri dish content was poured in wash basin and allowing the worms to move freely. The paralysis time and death time were recorded in terms of minute.

1.8. Phytochemical studies

The study also comprised preliminary phytochemical studies (qualitative and quantitative analysis) of aqueous and HAEMS (50%). The extracts were subjected to various qualitative tests such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, phytosterols, fixed oils, coumarins, diterpenes, flavonoids, proteins and amino acids etc. Since hydro-alcoholic extract showed more pronounced anthelmintic effect among all dosage forms therefore, HPLC finger printing of the hydro-alcoholic extract of ingredient of MSwas also carried out for the quantitative estimation of phytoconstituents accountable for anthelmintic activity i.e. flavonoid, tannin, alkaloid, phenols and saponin. The HPLC apparatus consisted of a Jasco Model PU 980 HPLC pump, UV- 975 tunable absorption detector and Clarity Lite software.

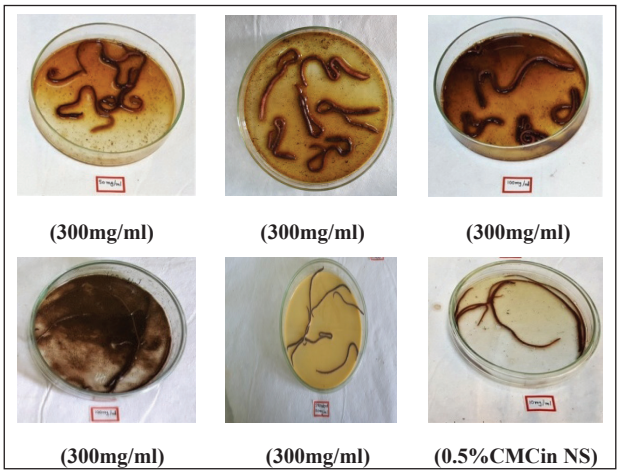

Figure 1: ***In vitro*** anthelmintic activity of various dosage forms of *Majoon Sarakhs,* standard and control.

Figure 2: Discolouration of Earthworm after death

2.8.1. *HPLC analysis of phenols*

The analysis was made in isocratic mode with the mobile phase acetonitrile and water in the ratio 7:3 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard gallic acid (100µg/mL) and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 254nm.

2.8.2. *HPLC analysis of flavonoids*

The analysis was made in isocratic mode with the mobile phase acetonitrile and water in the ratio 7:3 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard quercetin (400µg/mL) and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 272nm.

2.8.3. *HPLC analysis of tannins*

The analysis was made in isocratic mode with the mobile phase methanol and water in the ratio 5:5 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard tannic acid (100µg/mL) and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 270nm.

2.8.4. *Estimation of Saponins*

Total saponin determination was done using vanillin reagent. Different aliquots of standard saponin (1mg/mL) were taken in different test tubes and the volume was made upto 1mL with absolute methanol in all the test tubes. Later, 500µL of 8% vanillin and 500µL of 72% sulphuric acid was added in all the tubes and incubated at 60ºC for 10 minutes. After incubation, the absorbance was read at 544nm using a UV/Vis spectrophotometer. The sample was also processed in the similar manner by taking 1 ml of sample. The standard graph was plotted and the amount of saponin in each sample was calculated.

2. Statistical analysis

Statistical evaluation of the data was performed by one-way ANOVA followed by Tukey-Kramer Multiple Comparisons test. The results were expressed as mean ± SEM using Graph Pad Instat3 (n = 6). P<0.05 was considered significant.

3. Results

After observation of results it was noted that all dosage forms has shown anthelmintic effect at minimal dose (50mg/ml) except PFMS. Further all the dosage forms including Albendazole exhibited anthelmintic effect in concentration dependent manner. HAEMS has shown more pronounced effect as compared to other three dosage forms. Moreover, both aqueous and hydro-alcoholic extracts of the ingredients of MSwas found to be more potent than Albendazole. The anthelmintic activity of the extract was directly proportional to the dose. The activity confirms the dose dependency nature of the extract. In comparison to another dosage forms, PFMShas shown least response (Table. 1).

Phytochemical analysis of *majoon* and extracts revealed the presence of phytoconstituents responsible for anthelmintic activity. Since, HAEMS showed better effect among other dosage forms that is why it was further subjected to HPLC analysis, which quantified the amount of flavanoids (45.8 µg/ml), tannins (0.5 µg/ml) and phenols (2.86µg/ml). Saponin estimated by spectrophotometer was found to be 6.952mg/mL in the hydro alcoholic extract further justified the effect of HAEMS (Fig. 3-6).

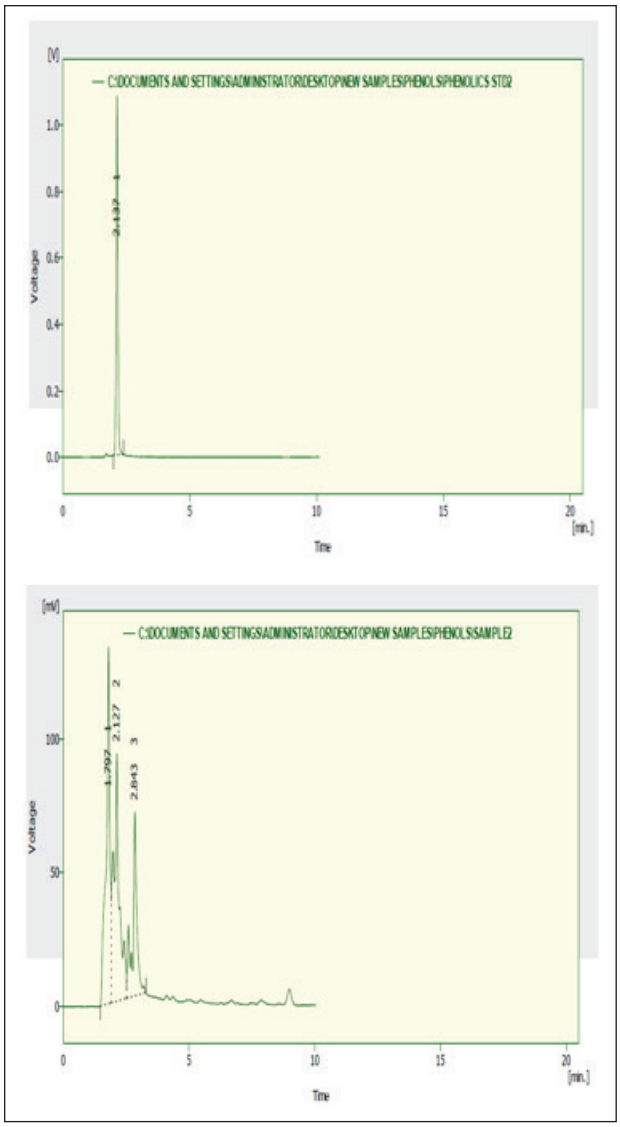

Figure 3: Graph of HPLC analysis of standard gallic acid and HAEMS for phenols

4. Discussion

Majoon Sarakhs ***is an important polyherbal Unani formulation which has been described for the treatment of Intestinal helminthiasis especially for round worm and Tapeworm. The present study has been suitably designed to investigate*** *in vitro* ***anthelmintic effect of ingredient of*** *Majoon Sarakhs* ***in various dosage forms including*** *majoon* ***itself at different concentrations on the adult earthworms (****Eisenia fetida****). Different*** *in vitro* ***and*** *in vivo* ***models are used for the evaluation of anthelmintic activity. Most of the screenings reported are*** *invitro* ***studies, using a number of worms as model such as Indian earthworm*** *Pheretimaposthuma, Ascardiagalli, Ascaris lumbricoids* ***etc. Several studies have already been published on*** *in vitro*

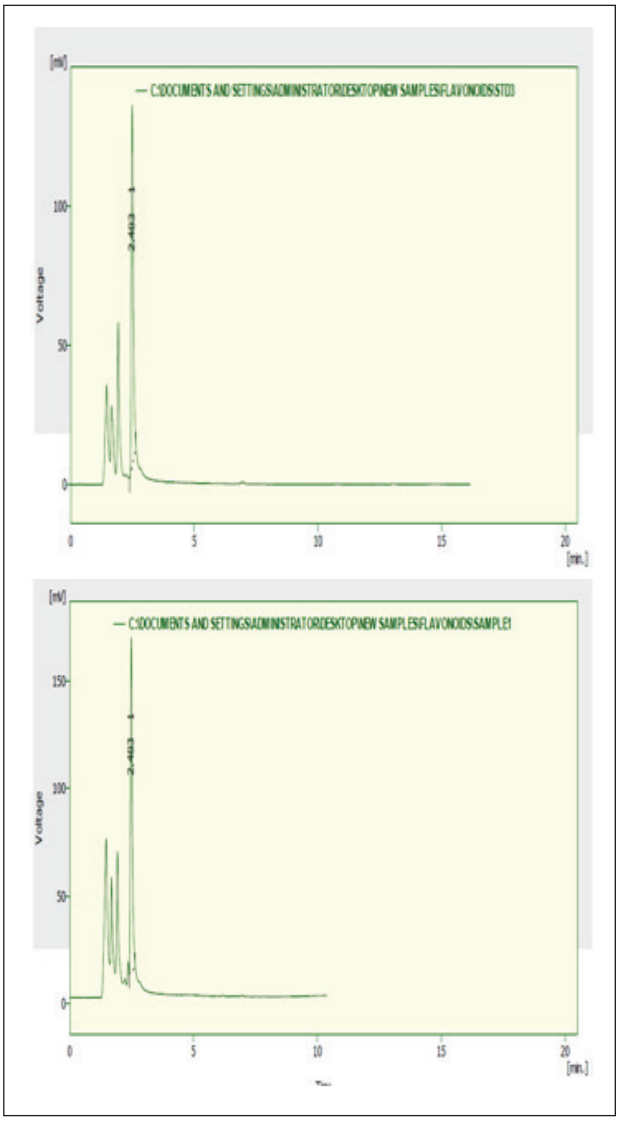

Figure 4: Graph of HPLC analysis of standard quercitin and HAEMS for flavanoid

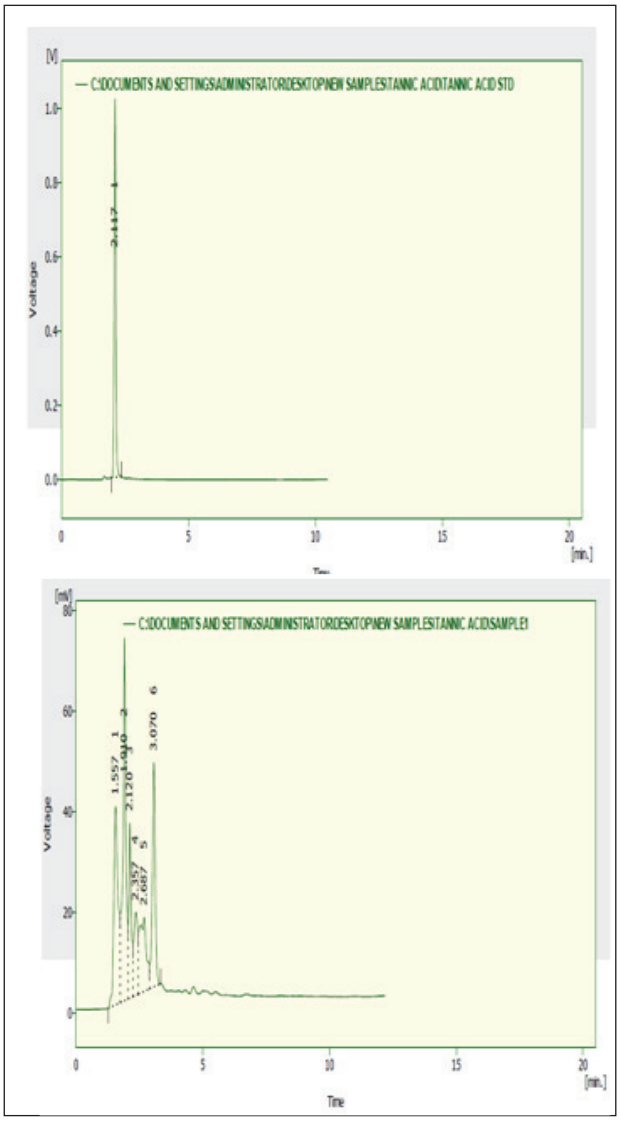

Figure 5: Graph of HPLC analysis of standard tannic acid and HAEMS for tannin

anthelmintic effect of medicinal plants on *Eisenia fetida*[25]***.Earthworms have the capability to move by peristalsis and clutch the surface by setae. Their outer layer is mucilaginous and composed of complex polysaccharides. This layer being slimy facilitates free movement of earthworms. Any harm or injury to the mucopolysaccharide membrane exposes the outer layer which causes irritation resulting in restriction of movement and further leads to paralysis and death of worms. Therefore, two parameters, time of paralysis and time of death in minutes were observed as an index of anthelmintic activity***[31].

After analysis of the results it was observed that each dosage form viz. HAEMS, AEMS, MS, PFMS caused paralysis ranging from loss of motility to loss of response to external stimuli, which eventually

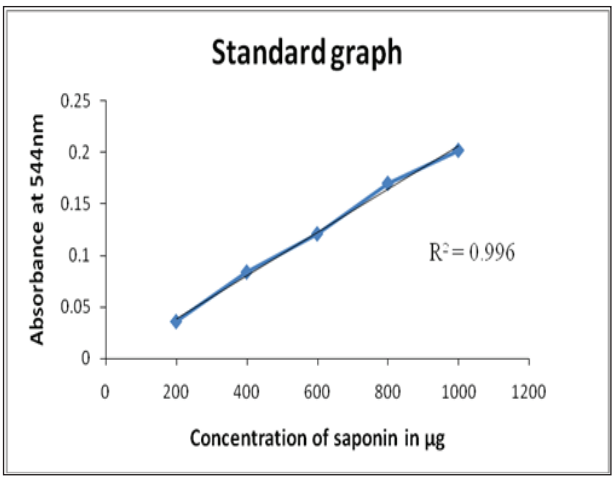

Figure 6: Graph of Saponin concentration in HAEMS sample

Table no. 1, 2. Comparative anthelmintic effect of *Majoon Sarakhs* and various dosage forms of its ingredients of on Indian adult earthworm.

Conc. (mg/ml)	Groups: Albendazole		HAEMS		AEMS	
	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM
50 mg/ml	87.5 ± 2.04	94.83 ± 1.35	59.00 ± 6.3***	77.67±6.85**	88.67 ± 7.13	117.83±2.06***
100 mg/ml	59.83 ±1.70**a**	79.67 ± 1.14**b**	32.00 ± 2.66**a**	35.83±1.47***a**	31.33±1.80***a**	39.17±2.09***a**
150 mg/ml	40.00 ±2.83**a**	74.00 ± 3.08 **a**	28.83±2.66***a**	31.50±2.55***a**	29.50 ± 3.49**a**	33.17±1.60***a**
200 mg/ml	32.67 ±3.71**a**	67.33 ± 4.18 **a**	17.33±1.65***a**	26.00±1.74***a**	20.33 ± 1.52**a**	26.33±1.56***a**
250 mg/ml	27.83 ±4.72**a**	66.17 ± 1.77 **a**	11.33±1.33***a**	19.33±0.91***a**	15.67 ± 1.33**a**	25.83±1.33***a**
300 mg/ml	16.50± 1.82**a**	30.17 ± 1.17**a**	8.67 ± 0.92***a**	18.67 ±2.33**a**	11.67 ± 1.33**a**	17.50 ± 0.72**a**

Table no. 2.

Conc. (mg/ml)	Groups: Albendazole		*Majoon Sarakhs*		PFMS	
	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM
50 mg/ml	87.5 ± 2.04	94.83 ± 1.35	114.83 ± 8.85	165.33 ± 2.01	166.83 ±4.63	202.33 ± 4.22
100 mg/ml	59.83 ±1.70**a**	79.67 ± 1.14**b**	48.33±6.42***a**	63.00 ± 8.85**a**	141.5 ± 3.61**a**	169.00 ± 6.01**a**
150 mg/ml	40.00 ±2.83**a**	74.00 ± 3.08 **a**	32.00 ± 3.43 **a**	43.00 ±2.77***a**	73.67 ± 2.51**a**	125.33 ± 4.30**a**
200 mg/ml	32.67 ±3.71**a**	67.33 ± 4.18 **a**	23.83 ± 2.86**a**	31.67 ±1.87***a**	41.50 ± 4.03**a**	86.17 ± 0.87**a**
250 mg/ml	27.83 ±4.72**a**	66.17 ± 1.77 **a**	18.17 ± 1.95**a**	25.33 ±2.45***a**	39.00 ± 1.86**a**	61.83 ± 1.51**a**
300 mg/ml	16.50± 1.82**a**	30.17 ± 1.17**a**	14.16 ± 2.14**a**	22.17 ± 1.79**a**	38.67 ± 3.38**a**	50.83 ± 1.25**a**

Control (0.5% CMC in NS)	Time of paralysis(min.) Mean ± SEM	Time of death (min.) Mean ± SEM
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Test used: One way ANOVA with Turkey compare all pairs of column. P<0.05 was considered significant.

HAEMS: Hydro alcoholic extract of *Majoon Sarakhs*,AEMS: Aqueous extract of ingredients of *Majoon Sarakhs*, PFMS: Powder form of *Majoon Sarakhs*.

HAEMS, AEMS, *MajoonSarakhs*, PFMSvs. Albendazole with respective concentration (*p<0.05, **P<0.01, ***p<0.001)

HAEMS, AEMS, PFMS, *Majoon Sarakhs*and Albendazole at 100, 150, 200, 250,300 mg/ml vs. 50mg/ml (**a**-p<0.001, **b**-p<0.01)

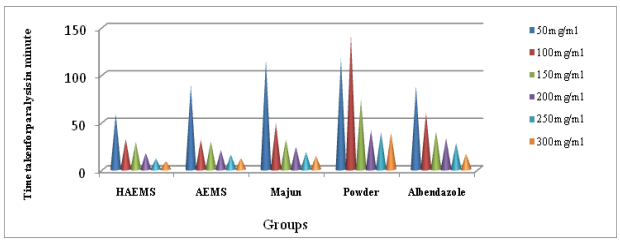

Figure 7: Graphical presentation of comparative effect of various dosage forms of ingredients of Majoon Sarakhs on time of paralysis of Indian adult earthworm

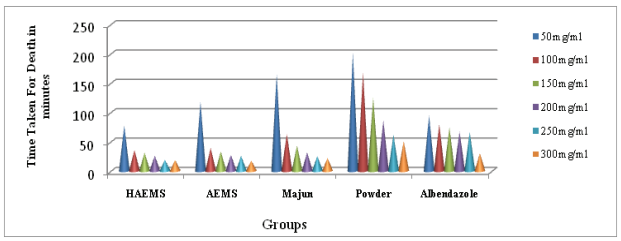

Figure 8: Graphical presentation of comparative effect of various dosage forms of ingredients of Majoon Sarakhs on time of death of Indian adult earthworm

progressed to death. The test formulation not only caused paralysis but also caused death within two hours at all concentration except PFMS at 50 and 100mg/ml. Besides, all the dosage forms (except PFMS) showed the anthelmintic activity at minimal dose of 50mg/ml. However, at high dose (300mg/ml) Majoon showed greater effect than Albendazole.

The anthelmintic activity of HAEMS when compared to Albendazole with respective concentrations, significant effect ($p<0.001$) on paralysis was observed at 50,150, 200, 250 and 300mg/ml but not at 100 mg/ml. Though, the test extract took very less time in paralysing the worms in comparison to Albendazole, statistically not significant. Further, the test extract showed significant ($p<0.01$) effect on mortality at 50mg/ml while highly significant ($P<0.001$) effect at all concentration (except 300mg/ml) when compared to Albendazole. Though, HAEMS at 300mg/ml took only 18.67±2.33min. to kill the worms while Albendazole took 30.17±1.17min, however, statistically not significant. When the different concentrations of HAEMS were compared to the first dose (50 mg/ml) strong significant ($p<0.001$) anthelmintic effect was observed at all the concentrations. However, hydro-alcoholic extract was found to be more effective than Albendazole. The anthelmintic effect /lethal effect was directly proportional to the dose of extract as the extract took less time to paralyse and kill the worms with subsequent increase in dose. The result evidently showed the concentration dependent effect of HAEMS.

The effect of AEMS on time of paralysis at 50, 150, 200, 250, 300 mg/ml concentration were not found to be statistically significant when compared to Albendazole at the same concentration, but time was found to be gradually decreased in comparison to standard drug. However, at 100mg/ml it showed highly significant effect ($P<0.001$) in comparison to Albendazole. The high significant effect ($P<0.001$) on mortality was observed at all concentration, but not at 300 mg/ml, yet it was found to be more potent than Albendazole.When the different concentration of AEMS were compared with first dose (50 mg/ml) significant ($p<0.001$) dose dependent anthelmintic effect was observed. The activity confirms the dose dependency nature of the extract. Further, the findings verified that the AEMS is more potent than Albendazole.

(After going through the papers on anti lithiatic activity it was noted that albendazole is used either in a single dose i.e. 100mg/ml or in different concentration same as test compound. Therefore, in the present study the various concentration of test formulation and albendazole was investigated so that the effect of each concertation can be compared directly .)

Majoon Sarakhs showedno statistically significant effect on the time of paralysis at all concentration whereas at 100 mg/ml it showed high significant anthelmintic activity ($p<0.001$) on the same parameter. However, MS paralysed the worm in much less time at all concentrations except at 50mg/ml. On the other hand, statistically significant effect was observed in the time of death at 100 mg/ml ($p<0.01$) and at 50, 150, 200, 250 mg/ml ($P<0.001$) but not at 300 mg/ml. The findings clearly showed that MS at 50mg/ml is not as effective as aqueous and hydroalcoholic extract at the same concentration. When different concentration of *Majoon Sarakhs* were compared with first dose (50mg/ml) strongly significant ($p<0.001$) anthelmintic effect was observed at all the doses. The results undoubtedly demonstrated the concentration dependent effect of *Majoon Sarakhs.*

On the contrary, PFMS at all the concentrations took much more time in paralysing and killing of earthworms. PFMS at 50 and 100mg/ml did not show significant anthelmintic effect as it took more than two hours in paralysing and killing of worms while at rest of the doses it took only one hour or less than one hour but was found to be less potent than Albendazole. On the basis of above findings it is verified that powder form of *Majoon Sarakhs* is not as much effective as other three dosage forms. When different concentration of the PFMS were compared to the first dose (50 mg/ml) it showed strong significant activity ($p<0.001$) at all concentrations. The result noticeably demonstrated the concentration dependent effect of PFMS.

All dosage forms viz. HAEMS; AEMS, MS at all doses and PFMS (only at higher dose) has shown significant anthelmintic effect in concentration dependent manner. Interestingly, hydro alcoholic extract showed more pronounced effect as compared to other three dosage forms and Albendazole. Moreover aqueous extract also exhibited much effect than standard drug. The order of activity was recorded as HAEMS > AEMS > Albendazole > MS > PFMS at lower dose (50mg/ml); the anthelmintic effect was found to be directly proportional to the dose; with the increase in concentration of drug a decrease in the time of paralysis and death of worms was noted. However, at high dose (300 mg/ml) *majoon* showed

greater effect than Albendazole.

Hydro alcoholic extract exhibited more strong effect among all dosage forms; it might be due to the higher concentration of the active principle present in this extract. As in the present study during standardization of extract the highest yield of extract was obtained in hydro-alcoholic solvent in comparison to water. The reason for variation in the yield of the aqueous and hydro-alcoholic extract of the ingredients of *Majoon Sarakhs* could be due to solubility of constituents as some are more soluble in organic solvents while others are in water and vice versa. In the present study, all the four ingredients of *Majoon Sarakhs* were extracted collectively in aqueous and in 50% ethanol. 50% ethanolic extract dissolves almost all alcohol and water soluble chemical constituents [32].

Anthelmintic drugs can reach target site in nematodes either by oral ingestion or by diffusion through the cuticle. However studies have shown that transcuticular diffusion is a common means of entry for non-nutrient and non-electrolyte substances in nematodes[33].It has also been shown that this route is predominant for the uptake of major broad-spectrum anthelmintics; benzimidazole, Levamisole and ivermectin by nematodes, cestode and trematode as opposed to oral ingestion. The variation in activity of the each dosage form might be due to the poor solubility of ingredients, particularly powder and *majoon* where crude ingredients are used and it is not possible to completely dissolve the phytoconstituents in water or normal saline in a very short time (1-2 hours). Moreover, it is quite interesting to note that there is remarkable difference between the response of *Majoon* and powder of ingredients of *Majoon Sarakhs*. *Majoon*has shown good anthelmintic activity than powder. It might be due to some chemical changes that took place in the ingredients during the preparation of *majoon*and its semisolid consistency might be the reason for the increased the solubility of the active components. But the interesting fact of this finding is that it's in agreement with the view of Unani physicians that worms get attracted towards sweetness and accept it more easily than any other taste[34],[35]. Further, powder is found to be more viscous due to bulkiness and poor solubility of drug in normal saline/water, which might hamper the absorption of drugs. Hence it took more time to show anthelmintic effect.

Benzimidazoles are widely used anthelmintic drugs. Thiabendazole, Oxibendazole, Mebendazole and Albendazole are the drugs used under this class. Albendazole being a lipophilic anthelmintic has a better ability to cross the external surface of the helminthes than the hydrophilic compounds. Transcuticular passive diffusion across the lipid component of the parasite cuticle is considered as the rate-limiting step in the process of drug absorption into helminths.[33]The mechanism of test drug is not yet fully understood but the anthelmintic activity as evident from the result could be attributed to the above mechanism up to some extent. However,in the present study both the hydro-alcoholic and aqueous extract were found more effective than Albendazole, this indicates that the extracts have a greater capability to cross the external surface of the helminthes in comparison to Albendazole. The reason behind the effectiveness of test extract is that, this polyherbal formulation is rationally designed by experienced Unani physicians in an appropriate ratio. Hence the different ingredient in one formula when combined may produce synergistic effect as they are enriched with a number of phytoconstituents. Thus the highest efficacy may be attributed due to the combined effect of phytoconstituents present in the ingredients of *Majoon Sarakhs. Baobarang* is one of the ingredient of *MajoonSarakhs*; its aqueous and hydro alcoholic extract showed significant anthelmintic activity[13] Essential oil of *Muqil*[23]and *Sarakhs* has also been documented for their larvicidal and pupicidal effect on the third instar larvae of *Corcyra cephalonica*[36]. Similar effect was also observed in the study of Diwedi et al.,[37] where alcoholic extract showed better anthelmintic effect than Albendazole. Likewise, Singh et al., (2011),reported synergistic effect of combination of hydroalcoholic extracts of *Zingiberofficinale* and *Curcuma longa*[38].Moreover, numerous studies reported comparable results and support the findings of the present study that medicinal plant extracts are powerful anthelmintics[13].

Unani medicine whether used as a single drug or compound formulation or in an extract form comprises of vast number of bioactive constituents such as alkaloids, flavanoids, saponins, tannins, oleoresin etc. Their action is dependent not only on single molecule but different bioactive molecules present in the drug that act synergistically to produce the desired effect. It is often difficult to determine which component is responsible for pharmacological activity. The anthelmintic effect of the test drug formulation may be due to the presence of these phytochemicals as *Sarakhs*(*Dryopteris filix mas*),[19]which contains flavone, dryopterin, tannin, triterpenoids and Phloroglucinol derivatives such as aspidin, deaspidin, and filixic acid which has phenol-like characteristics,

and is reported to have a strong anthelmintic especially taenicidal activity.[8]*Baobarang*(*Embeliaribes*)[24] and *Muqil*(*Commiphoramukul*)[23].also contain alkaloids, tannin, essential oil in good quantity. Moreover qualitative phytochemical analysis also revealed that *majoon*as well as aqueous and hydro alcoholic extract of the ingredients of *Majoon Sarakhs* contain carbohydrates, alkaloids, tannins, flavonoids, saponin, terpenes, and phenols. Further, HPLC finger printing of hydro-alcoholic extract also quantified the amount of flavonoid, tannin, phenols and saponin. Hence the presence of these phytoconstituents further justified the effectiveness of test formulation. Moreover hydroalcoholic extract showed rich amount of flavonoid, the more potent effect of hydroalcoholic extract might be due to the presence of flavonoid and tannin.

However, such *in vitro* data cannot be directly extrapolated to the more complex milieu in intestine of human body where many other physiologically occurring substances may modulate the effect on intestinal round worm. Therefore, it is suggested that these *in vitro* results should be confirmed *in vivo* with the aim to develop potent anthelmintic drugs. Further, characterization and isolation of the main active compound from the formulation are required that could be analysed for future studies.

5. Conclusion

On the basis of results and discussion it can be summarized from the present study that all the dosage forms of ingredient of *Majoon Sarakhs*have shown promising *in vitro* anthelmintic activity against adult Earthworm (*Eisenia fetida*) in concentration dependent manner. Among all the dosage forms, hydroalcohalic extract was found to be more potent, could be due to presence of maximum number of phytoconstituents, as revealed by qualitative and quantitative estimation of hydroalcoholic extract It can be concluded therefore, that *Majoon Sarakhs* and its extract has profound anthelmintic activity against tested worm species. It validates the claims of Unani physicians that *Majoon Sarakhs* is an effective anthelmintic agent. Further *in vivo* studies against different parasites species and clinical trials are needed for evaluating its potential therapeutic effect. However, the present study will be helpful as additional information to the scientific evidences regarding *in vitro* studies.

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