

Fourth Islamic Manuscripts Conference, Queen's College, University of Cambridge, 6-9 July 2008, Mehrnaz Azadi Boyaghchi, Conservation Department, PhD student, NMI, new Delhi,

The importance of using the extract of *Hanzal* as inhibitors in paper manuscripts

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Abstract

In Iran one kind of plant had been used, along with the sizing material, as an insecticide. Therefore it protects paper from later damage. According to the literature some artists recommended that the juice of the *Shahm-e-Hanzal* be used to make paper to protect from the damage of mice and insects. *Shahm-e-Hanzal*, with the scientific name of *Citrullus colocynthis* (Family: *Cucurbitaceae*), contains different principle constituents. Some of these principle constituents have the role of insecticides therefore those papers on which artists apply this juice along with starch, weren't good resource for biological factors in Persian manuscripts. The reason why some papers had remained without any damage is that the artists apply some (traditional preventive) protective material. Thus, by considering the importance of these medicinal plants and their principle constituents by isolating insecticidal parts of a plant the result will be more considerable. Moreover we can have a good replacement for chemical materials. Instead of using chemical materials that most of time are very toxic and expensive we can use some useful herbs like *Cirullus colocynthis*. In this article the importance of this kind of herb and the reasons of using of it in accordance with scientific survey will be introduced.

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“The importance of using the extract of Hanzal as inhibitors in paper manuscripts in Iran”

Introduction:

Some medicinal herbs have been used by Iranian artists, and they were adept at using these herbs through the ages. The aim of the research work is to introduce one of the herbs that has been used against insects and mice in paper manuscripts, to introduce chemical properties of this plant and the reasons of applying *Hanzal*'s juice. Hereafter in the article *Hanzal* will be known with the scientific name of “*Citrullus colocynthis* (L.) Schrad (Family: Cucurbitaceae)” There are effective substances (principle constituents) in plants that can be used to protect cultural heritage from serious damages. Therefore, a scientific study needs to be done on those plants which can be used to prevent deterioration of cultural heritage. This subject can be one of the priorities in conservation workshops and phytochemistry laboratories. Since these items of cultural heritage are sensitive to destructive parameters, their preservation and conservation have been very significant all over the world.

This paper will consider three areas:

- Why artists used it?
- What is *Hanzal*?
- How it can help to protect paper?

1- Why artists used *Hanzal/Citrullus colocynthis*?

In literature it has been mentioned that artists added the extract of *Hanzal* to the mucilage of rice before sizing the paper. In 1158 AD *Abobakr-e-jamali Yazdi* and in 1157AD *Hobaish-IbneIbrahim-e-Taflisi* had explained in *Farrokhtnameh* and *Bayanolsana-at* respectively about the preparation of sizing material and then emphasized on the addition of the extract of *Hanzal* (*Citrullus colocynthis*) to the sizing material to protect the paper from the harm of insects and mice.

Also *Abobakr-e-jamali* mentioned different sizing materials such as starch of rice or flour, gum tragacanth (*Katira*), along with mucilage of rice that they could be used. Different sizing substances were used according to the paper's requirement. In Iran, in traditional medicine the plant of Colocynth was an effective treatment. The same method for preparation of drugs used the extract of *Hanzal*. It is considerable that *Abobakr-e-jamali* and *Hobaish-IbneIbrahim-e-Taflisi* were scientist in natural science. Thus they were aware about the properties of this medicinal plant. In addition, in agriculture fields the powder of this plan has been used against insects and mice. The plant of *Hanzal* is one of the plants that has never been damaged by insects in agriculture field in its whole life. It has never been a good source of food for insects and animals like mice in agricultural fields. In the region of the growth of *Hanzal* the local people call it "rat poison".

1-2- The role of sizing in paper:

The paper was sized:

- to improve the mechanical strength of the paper
- to make it more resistant to moisture damage
- for easy writing or painting
- to smooth the surface
- for a nonporous finish

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2- What is Hanzal?

2-1- Scientific Description of *Hanzal/ Colocynth*

2-1-1- Synonyms

Bitter apple; Bitter cucumber; Bitter gourd; Colocynth pulp; Hendeveane gorgi (Persian), Hanzal (Arabic), Indrayan (Hindi); Colocynthis;, Fructus Colocynthis. (Figure 1).



Figure 1: The *Citrullus colocynthis* on the ground

2-1-2- Biological source

Colocynth is the dried pulp of the unripe but fully grown fruit of *Citrullus colocynthis* Schrad (Family: Cucurbitaceae).

2-1-3- Habitat

The plant is found in Syria, Cyprus, Sudan, North Africa, Spain, India and Iran. (Figure 2,3).

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Figure 2 Dehmolla in Iran



Figure 3: The *Citrullus colocynthis* on the ground with scale

2-1-4- Collection

The plant is a perennial herbaceous vine. The collected fruits are peeled to separate the epicarp and immediately dried in the sun. (Figure 4, 5).



Figure 4: Transverse section of the *Citrullus colocynthis* fruit



Figure 5: The dried fruit of *Citrullus colocynthis* on the ground

2-1-5- Morphology

The fruit is almost a globular berry, 4-10 cm in diameter. The peeled fruits are 4-8 cm in diameter, subspherical, nearly white, light in density and show sometimes small patches and impressions due to cuts made by a knife. The transverse section of the fruit shows three segments, divided by the radiating placentas, with seeds attached to the internal margins. The pulp is light, pithy and spongy, easily broken and is white or light yellow in color. (Figure 6,7).

Flat, ovoid seeds, 200-300 in each fruit, are present, which are compressed, brown or orange in color, one end somewhat pointed with rounded margin, 7mm long and 4.5 mm wide. The fruit is odorless and taste is very bitter.



Figure 4: The morphology of *Citrullus colocynthis*



Figure 5: The size and the shape of the fruit of *Citrullus colocynthis*

2-1-6- Chemical constituents

Colocynth contains an ether-chloroform soluble resin, a phytosterol glycoside, citrullol, pectin, colocynthein, albuminoids and other glycosides. The glycosides on hydrolysis form cucurbitacin E (α -elaterin), and cucurbitacin L (dihydroelatericin B). Choline and two alkaloids have also been isolated from the drug.

The other compounds present in colocynth are a bitter oil 'citbittol', glucose, α -spinasterol, citrullol, cucurbitacins B, I, E-2, J, elateridine, hexanor-cucurbitacin I, its 16-O-acetyl derivative and 2-O-glucoside, cucurbitacin L glucoside, hentriacontane, *n*-octacosanol and 1,26-hexacosaneddiol.

The fruit peel possesses 11, 14-dimethylhexadecan-14-ol-2-one, 10,14-dimethylhexadecan-14-ol-2-one, *n*-alkanes, lauric, myristic, palmitic,

Fourth Islamic Manuscripts Conference, Queen's College, University of Cambridge, 6-9 July 2008, Mehnaz Azadi Boyaghchi, Conservation Department, PhD student, NMI, new Delhi, hexadecenoic, stearic, oleic, linoleic and arachidic acids, hexadecanol, octadecanol, eicosanol, docosanol, tetracosanol, hexacosanol, methyl heptenone, citronellol, citronellal, phenylethyl alcohol, methyleugenol, docosanyl acetate, 10,13-dimethylpenta-13-decen-1-ol. (Figure 8)

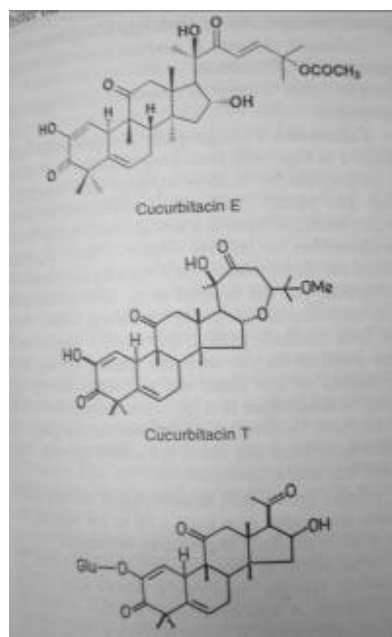


Figure 6: Cucurbitacins of colocynthis

2-1-7- Uses

As a medicinal plant, Colocynth is a very powerful cathartic. Cucurbitacin E is reported to possess anticancer activity. In agriculture fields it is used against mice and insects. Also its use was recommended to prevent manuscripts from the attack of mice and insects.

3- The role of *Hanzal/Citrullus colocynthis* in paper

To understand about the role of *Hanzal* in paper and to find the same features that in literature had been mentioned about *Hanzal* some tests has been done on this plant.

3-1- survey on pesticide materials of *Citrullus colocynthis* (L.) Schrad.

The present study was designed to investigate whether this fruit possesses insecticidal or fungicidal effects or possible protective action or not.

The materials prepared from this plant extract were subjected to biological assays like:

- Antifungal activity
- Insect repellent activity
- Mice repellent activity

3-1-1- Laboratory examination on *Citrullus colocynthis* (L.) Schrad

a) Preparing *Citrullus colocynthis* Extraction for measuring pH

To prepare the water extraction, dry fruit of *Citrullus colocynthis* was ground. To this powder distilled water was added. Then it was left for 24 hours. After taking the water extraction of *Citrullus colocynthis* its pH was measured. The pH was 5.22. It means that *Citrullus colocynthis* has acidic features. (Figure 9-12).

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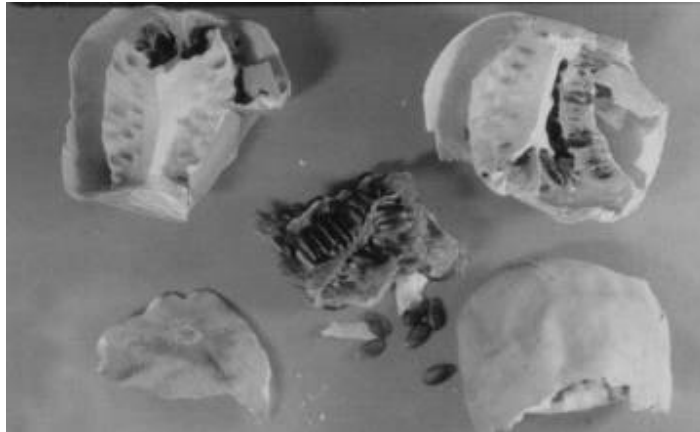


Figure 7 removing seeds from the plant to weigh the proper amount of plant

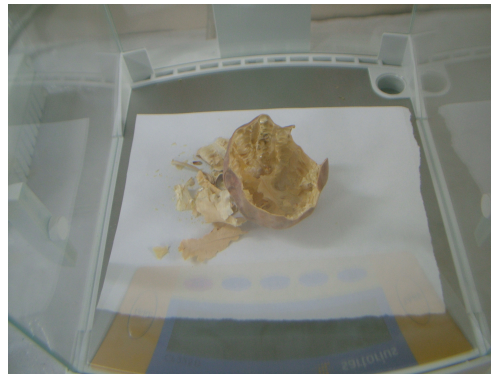


Figure 8: Weighting the proper amount of the plant



Figure 9: Mixing with water



Figure 10: Filtering

b) Extraction of Hanzal/Citrullus colocynthis to survey on pesticide materials of Hanzal

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The simplest extraction processes employed was extraction with water. The sample should be coarsely fragmented; enough to pass through a 3 mm sieve would be adequate. After grinding and weighing, the adequate amount of sample was steeped for 24 h (for up to three times) in solvent in the beaker that contained distilled water, and then the extract materials were collected and pooled. After a triple-solvent extraction, the remaining marc did not contain valuable material. Then the extract was concentrated to get less volume of extraction. (Figure 14-16).



Figure13: Grinding



Figure 14: sample was steeped in distill water

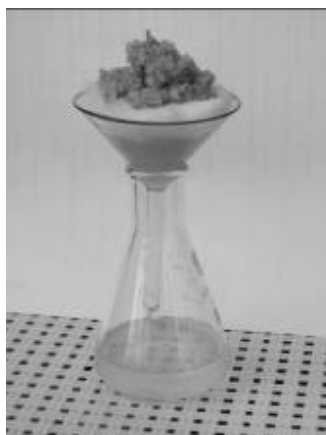


Figure 15: filtering of solution and remaining marc



Figure 16: the concentrating of solution

c) The preparation of mucilage of rice according to (*Farrokhtnameh and Bayanolsana-at*)

Materials required are rice, water, muslin and a big pot.

- First fill the pot with water; then add rice.
- Put the pot on a fire. The mixture must be boiled until the rice completely cooks.
- Remove from the flames.
- To remove any lumps, strain the entire mixture through a muslin cloth into a large separate bowl.
- Mixture is ready to use as a sizing material
- Paper will be sized one or two times.
- It will be left to dry
- After drying paper will be burnished

To avoid attacks from insects and mice, the extract of *Hanzal* will be added.

c) Preparation of paper sheet

Materials used:

- Concentrated extraction
- Handmade paper
- Mucilage of rice

Hand made paper were impregnated with active ingredients

- The paper was treated with a solution of extraction of *Hanzal* in water at varying concentrations. The extracted drug was diluted 1X, 2X, 3X, 4X, 5X, 6X diluted.

- Treated with mucilage of rice
- Treated with mucilage of rice and varying concentrations of Hanzal. Extracted drug was diluted 1X, 2X, 3X, 4X, 5X, 6X diluted.
- Untreated hand made paper (hand made paper without adding any sizing or drug).

3-1-1-1- Mice repellent activity

Testing of prepared traditional materials (paper sheet sample)

After preparing paper sheets, they have been tested in Albino Wister rats in a propylene case in the Laboratory. The mice level of interest was evaluated. When papers were kept in the mice's environment, they had different reaction to different samples. After smelling those samples that include *Hanzal* they rejected eating them. (Figure17,18). Assessment of mice repellent activity of paper samples with *Hanzal*, Mucilage of rice and mixture of *Hanzal* and mucilage of rice in varying concentrations has been shown in following table. (Table 1)



Figure 17: paper samples have been tested



Figure 18: mice environment/ Albino Wister rats in propylene case

Table1: The level of interest of mice

| No | Sample dose | The level of interest of mice |
|----|--|-------------------------------|
| 1 | <i>Hanzal</i> | No interest |
| 2 | Mucilage of rice | Interested |
| 3 | <i>Hanzal</i> + mucilage of rice in varying concentration | No interest |
| 4 | Hand made paper | No interest |

3-1-1-2- Insect repellent activity of paper sheets impregnated with extracts

- To assess insect repellent activity against *Hanzal* by application of paper sheet impregnated with extract. These paper sheets kept in an insect environment. It was observed that the presence of extract is sufficient for repelling insects in the case of *Hanzal*. According to the literature *Hanzal* was recommended against the house fly (*Mosca domestica*). The same result has been observed during examination. The result of the test has been shown in the table. (Table 2)

Table 2: The level of interests (house fly "*Mosca domestica*")

| No | Sample dose | The level of interest of insects |
|----|--|----------------------------------|
| 1 | <i>Hanzal</i> | No interest |
| 2 | Mucilage of rice | Interested |
| 3 | <i>Hanzal</i> + mucilage of rice in varying concentration | No interest |
| 4 | Hand made paper | No interest |

3-1-1-3- Antifungal study of *Hanzal*

Materials and Methodology

Microbiological medium (Potato Dextrose Agar) for fungal growth

Potato dextrose agar (PDA) formulation

| | |
|-----------------|--------|
| Potato (peeled) | 200 g |
| Dextrose | 20.0 g |
| Agar | 15.0g |
| Distilled water | 1000ml |

Preparation of Potato dextrose agar (PDA) medium

The skin of potatoes were peeled off and cut into small pieces and boiled (200g) in 500ml of water, till a glass rod penetrates them. Filtered through cheesecloth,

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Microbial strains:

Fungal strains such as Aspergillus niger MTCC 281 and *Aspergillus flavus* MTCC 277 were Microbial Type culture Collection and gene bank of IMTECH (Institute of Microbial Technology, India) and *Penecillium citrinum* NCIM 768 was *collected* from Nation Collection for Industrial Microorganism, Nation Chemical Laboratory, Pune, India.

All the fungal strains were maintained on potato dextrose agar medium and subcultured in every one-month interval.

Anti fungal study

Active phytochemicals from plant was extracted with water and concentrated. Concentrated plant extract was diluted to 1X, 2X, 3X, 4X, 5X and 6X.

Microbiological Potato dextrose agar (PDA) medium was prepared according to standard procedure. Spore suspensions of different fungal strains were made in sterile distilled water containing TWEEN 80 (a surfactant).

Solid PDA medium was spread in Petri dish and fungal spore suspension was spread over agar surface and bores of 6 mm diameter were made. To each bore drug solution of different concentration were added and kept for drug diffusion

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The results of fungal study:

Out of all the fungal strains only *Aspergillus flavus* growth was inhibited by the plant extract (figure 19). However different dilution of plant extract shows different zone of inhibition. Plant extract such as 3X, 4X, 5X, 6X dilution only exhibit the fungistatic activity against *Aspergillus flavus* strain.

Fungal zone of inhibition was found to be 1.5 cm for 3X dilution (Figure 20), whereas 4X dilution shows 1.8 cm and 5X and 6X dilution shows 1.7 cm zone of inhibition.

The conclusion of Anti fungal study:

Drug with higher concentration such as 1X and 2X did not exhibit any fungi static activity against *Aspergillus flavus* strain this might be due to poor diffusion of extract through agar. However higher dilution such as 3X, 4X, 5X, 6X shows good fungi static activity.



Figure 19: Fungi static activity of Hanzal Plant extract against *Aspergillus flavus* strain

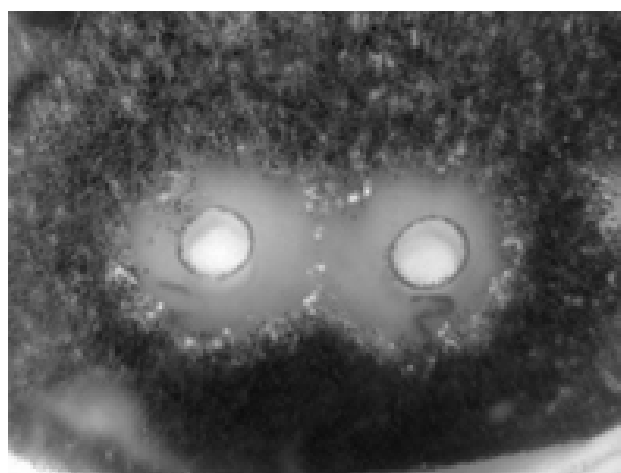


Figure 20: Fungi static activity of Hanzal Plant extract (3X dilution) against *Aspergillus flavus* strain

Discussion

For mice repellent activity the samples of Hanzal, mucilage of rice, Hanzal in combination of mucilage of rice in varying concentration and hand made paper were given to hungry mice for one hour. The mice showed interest for feeding on mucilage of rice only. For other samples they did not show any interest.

For insect repellent activity the Samples of Hanzal, rice mucilage, Hanzal in combination of rice mucilage in varying concentration and hand made paper were exposed to adult house *Mosca domestica* for 24 hours. It was observed that the house flies showed interest only in the mucilage of rice, and there was no interest for the other samples.

Antifungal study of Hanzal showed that the drug is effective against *Aspergillus flavas* but it is not effective against *Aspergillus niger* and *Penicilium setrium*. Also the diluted solution has better fungistation activity than concentrate drug solution.

Conclusion:

Hanzal as a natural product was designated to be effective against biological factors. Based on the result of different tests and the principle constituents of *Hanzal* it can be concluded that this drug has some active compounds that play the role of inhibitors. Here we can mention those active compounds that will work against insects, mice, and fungus activity. One of the most important compounds is glycosides and another compound is alkaloid, and the other is chloroform. They are present in the fruit of *Hanzal*; moreover, other principle constituents such as cucurbitacin E, cucurbitacins B, I, E-2, J, elateridine, cucurbitacin I, 16-O-acetyl derivative and 2-O-glucoside, cucurbitacin L are very active against insect mice and fungus.

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It seems to that it is possible to use *Hanzal* as a pest control. The method can be different from that which has been mentioned in literature. This plant, except glycosides and alkaloids, contains chloroform that makes it possible for us to use it for fumigation methods. Also it is feasible to take the essence of this drug.

Future plan:

Since the permanence of the chemical combination of plant is very important, it needs to be tested to determine how long a plant or the extract of plant is durable. Therefore, the duration of the effects of plant extract will be studied. Also the *Hanzal* will be studied. Also the possibility of taking the essence of this drug to use against insect will be studied.

Acknowledgment

Let me first thank the University of Hamdard, New Delhi, India, in particular the Faculty of Pharmacy, Prof. Dr. Shahid Ansari and Prof. Dr. Mohammad Ali who permitted me to work in their Laboratory (Pharmacognosy and phytochemistry labs) for my research. They have made it possible for me to complete this work. I would like to thank Dr. Panda in Biotechnology Laboratory in Hamdard University for antifungal study. I am also thankful Mr. Emtiyaz and Mr. Shoaeb for their co-operation in Pharmacognosy and phytochemistry labs. At last but not at the least my thanks also extended to TIMA for their support of my presence in the conference.

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