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Phytochemical and physicochemical evaluation of *Mocharasa* with reference to adulteration

Research Article

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Abstract

Introduction: Vegetable gums are used in medicine since ancient times. *Mocharasa* is an extensively used vegetable gum, used in formulations like *Piccha basti*. It is obtained in very small quantity from the plant source, but the use is extensive which is fulfilled by the substitutes or adulterants similar in color and appearance e.g. gum of *Moringa oleifera*. Aims and objectives: The present work aims to identify common adulteration of *Mocharasa* i.e. Moringa oleifera gum with the help of various organoleptic, physicochemical, phytochemical and microscopical studies and their comparison with each other for the identification of adulterant. Materials and Methods: Specimens used were authenticated *Mocharasa* sample, marketed sample of *Mocharasa* suggested as possibly adulterants like *M. oleifera* gum by National Institute of Science Communication and Information Resources, New Delhi and self-collected gum of *M. oleifera*. Comparative study was conducted for identification of adulterants with reference to organoleptic properties and pharmacopoeial tests. Discussion: The study revealed that marketed samples of *Mocharasa* were having similar physicochemical, phytochemical, and microscopical properties to that of Moringa gum as compared to the genuine *Mocharasa* sample. Conclusion: In the present study *Mocharasa* gum was found to be substituted or adulterated by Moringa gum.

Key words: Adulteration, microscopic study, *Mocharasa*, physicochemical study, phytochemical study

Introduction

Use of herbal medicines in India represents a long history of human interactions with the environment, right from the pre-Vedic and Vedic ages (5000 years back)(1). Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases. A vast knowledge of how to use the plants against different illnesses is now available [2]. The medicinal value of plants depends on the chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds, which are used as anti-bacterial, anti-fungal and anti-ageing agent depending on their compositions [3].

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi, and herbivorous mammals [4]. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total [5]. These compounds may be present in any part of plants such as leaves, flowers, fruits, roots, bark, gums, etc. so various plant parts are used according to the presence of these bioactive compounds and various formulations are prepared.

Mocharasa is a vegetable gum used in the specialized Ayurvedic treatments like *Piccha basti, Kshira basti*[6] etc., which are indicated in the treatment of *Pravahika* (dysentery) and *Atisara* (diarrhea) [7] It is derived from a lofty, deciduous huge tree, having hard prickles on the bark of young stems and branches; and described to be obtained from the natural wounds caused as a result of a functional disorder of the plant or decay by insects. It is obtained from the bark mostly in summer and dried, and then it is known as Semul-gum or *Mocharasa.* The dried gum is light brown in color, resembling the galls, and gradually becomes opaque and dark brown. The gum does not exude from artificially made wounds on healthy bark [8]. This exuded gum is obtained in very small quantity. It is used in the treatment of *Raktatisara* (diarrhea), *Pravahika* (dysentery), etc. It is also indicated in large number of formulations such as *Laghugangadhara Ras*, [9] *Kutajashtak kwatha,*[10] *Kutajashtakavaleha,*[11] and *Kutajavaleha,* etc. used in various diseases.

Adulteration of Vegetable Gums

There are wide number of plant species yielding gums that are, many times, very similar in color, taste and appearance. So, vegetable gums are largely substituted or adulterated, by similar looking gums. Adulterants used in crude plant products are mostly the substances that are similar in color and structure to the original substance, that cannot be separated easily and are heavier in weight, cheaper and easily available [12].

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Ayurvedic drugs are used as whole drugs and not as active ingredients only so these drugs are studied with the modern techniques and standard monographs are developed but along with the standardization, study of adulteration of each and every species is also utmost important. There is a need to set up a mark line between the original substance and the possible adulterants by using advanced studies like physicochemical, phytochemical tests for quality assurance of the raw materials.

Mocharasa is adulterated or substituted by gums of the similar species or similar appearing gums containing the same color, taste, and insolubility, for, e.g. gum of *Moringa oleifera*.

Mocharasa available in market shows wide variation in appearance and composition or its constitution. In the present study, *Mocharasa* procured from different markets of various regions of India revealed wide variations in appearance, color, hardness and structure, etc. [Figure 1, a-c].

Figure 1: Showing samples selected for the study

(a) *Mocharasa* authenticated sample labeled as A_1 sample

(b) *Mocharasa* sample procured from market, labeled as A_2 sample

(c) *Moringa oleifera* gum collected from plant source, labeled as A_3 sample

Aims and Objectives

1. To study the authenticated, marketed sample and possible adulterant, Moringa gum (which is often found added to the marketed samples so considered as the third sample for comparison), with various organoleptic, physicochemical, phytochemical, and microscopic studies.
2. To compare the results obtained for the samples with each other for identification of the adulterant, if any, by the above tests.

Materials and Methods

Seven samples of *Mocharasa* were collected for standardization study; out of which two samples were collected from plant source and were authenticated by the local botanist as gum of *Bombax ceiba* syn. *Salmalia malabarica, i.e. Mocharasa* and the other five samples were procured from local markets of Dehradun, Amritsar, Jamnagar, Akola, and Kolhapur [Figure 2].

Figure 2: Showing various samples of Mocharas

All these samples were sent to the Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi, (NISCAIR) for authentication.

But, out of these samples only Dehradun market sample was original *Mocharasa* and the others were not *Mocharasa* samples, but were suggested to be possible adulterant like gum of *M. oleifera.* Therefore, in order to validate the identity of these marketed samples of *Mocharasa*, three samples were selected - (1) sample procured from **Dehradun identified as original *Mocharasa*** by NISCAIR (labeled as A_1) (2) *Mocharasa* sample procured from **Jamnagar, unidentified as original *Mocharasa*** by NISCAIR, but purchased from market as *Mocharasa* (labeled as A_2) and (3) gum of ***Moringa***

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oleifera collected from plant source was taken (labeled as A_3). A comparative study was conducted for identification of adulterants by organoleptic properties and Pharmacopoeial tests.

Following tests were applied: (1) appearance, (2) organoleptic characters, (3) physicochemical tests, (4) swelling index, (5) alcohol-soluble extractive, (6) water -soluble extractives, (7) total ash values, (8) gas chromatography, (9) physicochemical tests as applicable, (10) powder microscopy, and (11) swelling index.

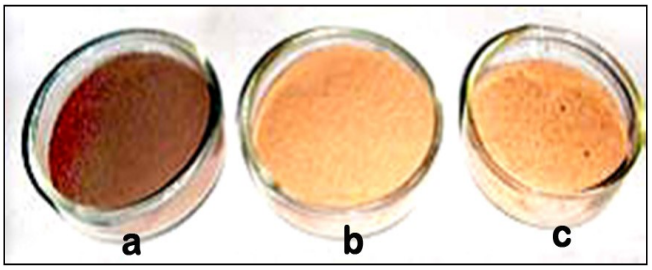
Observations and Results

The characters obtained were as follows:

(1) **Appearance**: The sample A_1 indicated a dark brown resinous mass, blackish brown in color, light in weight and fragile and opaque. On the contrary, the sample A_2 and the adulteration sample A_3, i.e. *Moringa oleifera* gum were found to be reddish brown in color, heavier, stout, and hard. The photographs [Figure 1 a, b &c] show a difference in appearance between sample A_1 and the sample A_2. The sample A_2 was similar in external appearance to *Moringa* gum i.e. sample A3 [Figures 1b and 1c].

Powdered samples of A_2 and A_3 were very similar in color and appearance but A_1 sample was dark brownish in color [Figure 3].

Figure 3: Powdered sample – (a) A_1 sample, (b) A_2 sample, and (c) A_3 sample



The genuine sample collected from the plant source was free from foreign matter, but samples collected from market both A_1 and A_2 contained very high percentage of foreign matter [13].

(2) **Organoleptic characters**: Table 1 shows the organoleptic characters of the three test drugs, which revealed that (a) sample A_1 appeared as dark brown resinous mass, blackish brown in color, light in weight and fragile; (b) the sample A_2 was reddish brown in color, heavier, stout, and hard; (c) the sample A_3, i.e. *Moringa oleifera* gum, were reddish brown in color, heavier, stout, and hard; and (d) the sample A_1 appeared opaque but the market sample and A_2 and A_3 appeared semitransparent.

Sample A_3 and the sample A_2 were similar in color, weight, fragility and structure. These are totally different in the sample A_1.

Table 1: Organoleptic characters of three test drugs

Characters	A_1 sample (Authenticated sample)	A_2 sample (Market sample)	A_3 sample (*Moringa* gum)
Color	Dark brown	Reddish brown	Reddish brown
Odor	Odorless	Odorless	Odorless
Taste	Astringent	Astringent	Astringent
Weight	Light	Heavy	Heavy
Structure	Hollow galls	Stout	Stout
Fragility	Fragile	Hard	Hard
Color of powder	Dark brown	Brown	Brown
Color of 10% aqueous extract	Dark brown	Reddish brown	Reddish brown
Transparency	Opaque	Semi transparent	Semi transparent

Physicochemical tests

(3) Swelling index: The swelling index test [14] was performed as per the Ayurvedic pharmacopoeia, which is the suggestive test for Mucilage content, and was found to be 4 ml in the sample A_1 but was twice in the A_2 and A_3 showing exceeding Mucilage content as compared with the original sample (table 3). This proved the similarity in Mucilage content of A_2 and A_3, and its difference from the A_1 (authentic sample).

(4) Alcohol-soluble extractive: The procedure performed as per the Ayurvedic pharmacopoeia showed an alcohol-soluble extractive [13] of 25-26% in the sample A_1, but was very less in the sample A_2, i.e., 15.952% and 6.06% in A_3(table 3). This shows that the difference in the alcohol-soluble part or constituents were more in the sample A_1 than in the A_2 and the sample A_3.

(5) Water-soluble extractives: Water-soluble extracts [13] were, on the contrary, very less in the sample A_1, and were about four to five times more than that of the sample A_1 in the sample A_2 and A_3 (table 3). This shows that water-soluble constituents were present more in the A_2 and the A_3 sample.

From the extractive values tests, it was clear that the A_1 sample was more soluble in alcohol and less in water, and is exactly opposite in case of the A_2 sample and A_3 sample. Thus, from the extractive values, it is clear that the A_2 sample has different constituents than the A_1 sample, which are more soluble in water than in alcohol.

(6) Total ash values: Total ash values [13] suggestive of inorganic contents in the crude drug were 2.69% in the A_1 sample and 3-4% in the A_2 sample and A_3 sample. Acid-insoluble [13] and water-soluble ash values [13] were not significant. (Table 3)

(7) Gas chromatography: From Table 2, gas chromatographic analysis [14] of the A_1, A_2 sample and

A_3 samples showed difference in retention time but similar number of peaks, which may be due to a difference in the concentrations of the constituents. Some peaks were similar in all the three samples, which may be due to similar constituents like tannin, Gallic acid, mucilage, etc. which are commonly present in gums. [Figure 4-6].

Table 2: Gas chromatography of all the three samples

Comparison of Peaks at retention time within the samples		
A_2 sample (market sample) Retention time(min) /T_R (min)	**A_1 sample (Authenticated sample) T_R(min)**	**A_3 sample (Moringa sample) T_R(min)**
7.040	7.043	7.107
7.853	7.883	7.890
8.393	8.403	8.420
9.433	8.667	8.683
9.877	9.473	9.900
11.930	9.890	11.950
15.277	11.937	15.340
	15.323	

Figure 4: Gas chromatography of A_1 sample (Dehradun authentic sample)

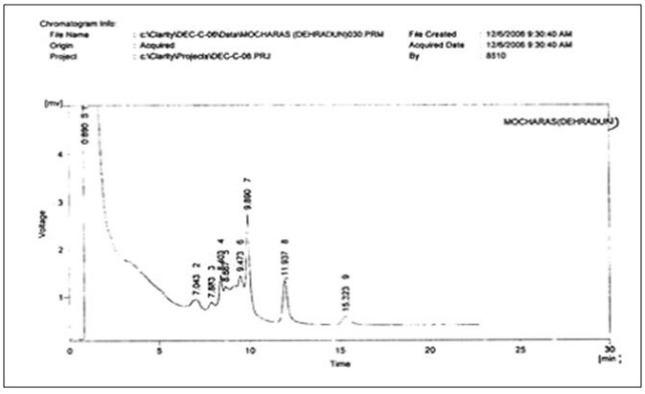


Figure 5: Gas chromatography of A_2 sample (Market sample)

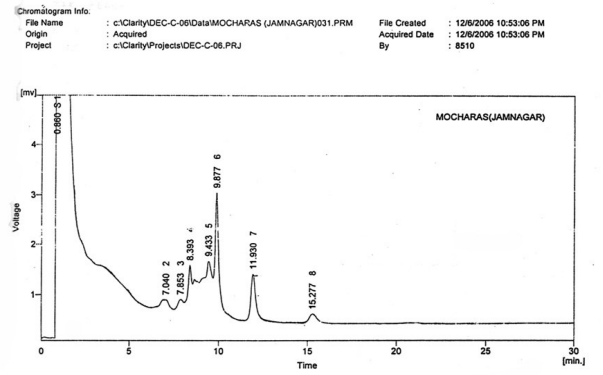
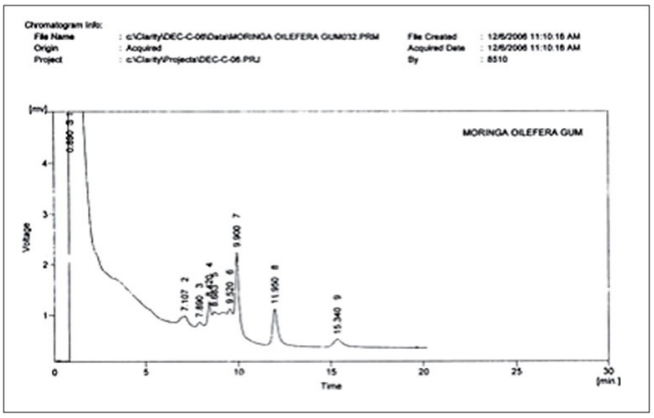


Figure 6: Gas chromatography of A_3 sample (Moringa gum)



(8) Tannin test: Tannin test [13] was performed as per the Ayurvedic pharmacopeia, and was found to be positive in all three samples. This may explain the similarity in some peaks of gas chromatography of all the three samples.

(9) Powder microscopy: Figures 7-12 shows the photographic findings of powder microscopy of powdered drugs of all three samples. Results are shown in Table 4.

Table 4: Powder microscopy of powdered drugs of all three samples

Characters	**A_1 Authenticated sample**	**A_2 Market sample**	**A_3 Moringa gum**
Mucilage cells	Mucilage cells in abundance	Mucilage cells in abundance	Mucilage cells in abundance
Types of fibers protein fibers and carbohydrate fibers	Protein fibers present carbohydrate fibers present	Protein fibers present	Phloem fibers in abundant quantity
Starch	1-2 granules	Starch scanty	Visible more than that of A.S.
Xylem vessels	Not visible	Very few	Pitted walled

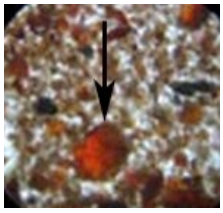
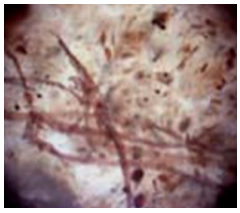
Figure 7: Mucilage cells (arrow) of A_1 sample (Mocharas genuine)	**Figure 10:** Phloem fibres of A_3 sample (Moringa gum)
	

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Figure 8: Mucillage cells (arrow) and xylem vessels (arrow head) of A_3 sample (Moringa gum)	**Figure 11:** A_1 sample (Genuine Mocharas, no stain used)

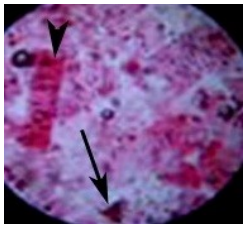
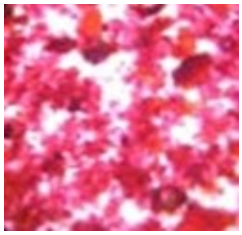
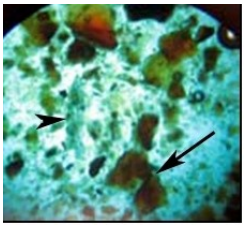
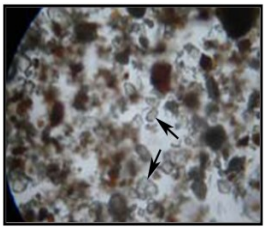

	

Figure 9: Unlignified fibres of A_1 sample (Mocharas genuine)	**Figure 12:** A_1 sample (Genuine Mocharas) stained with Sudan red, showing dark mucillage
	

From Table 4, (a) the mucilage cells [15] that are always present in gums were found in all the three samples, (b) starch granules may be the adulterant mixed, and was found to be more in A_3 sample, (c) xylem vessels [16] were present only in A_3 sample and very few in A_2 sample but totally absent in the A_1 sample, showing the similarity of the two samples and dissimilarity with the A_1 sample.

Discussion:

From Table 5, the organoleptic properties of all samples were as follows

Table 5: Comparative characters of all the three study samples

Characters	A_1 sample	A_2 sample	A_3 sample
Color	Dark brown	Reddish brown	Reddish brown
Odor	Odorless	Odorless	Odorless
Taste	Astringent	Astringent	Astringent
Weight	Light	Heavy	Heavy
Fragility	Fragile	Hard	Hard
Structure	Hollow galls	Stout/solid	Stout/ solid
Color of 10% extract	Dark brown	Reddish brown	Reddish brown
Swelling index	4 ml	8 ml	8 ml
GC (no. of peaks and RT in minutes)	7; 15.227	8; 15.225	7; 15.340
Powder microscopy xylem vessels	Not present	Very few	Pitted wall

GC: Gas chromatography, RT: Retention time

(1) Organoleptic characters:

The organoleptic characters of the three samples suggested that the A_1 (authenticated) sample is hollow and fragile, the A_2 sample (market sample) and the A_3 sample (adulterant) are stout, hard and heavier in weight, which is a significant difference in appearance of the samples. The original plant collected *Mocharasa* is always found to be hollow, fragile and light in weight. The above results showed that the A_1 (authenticated) sample was similar to the original plant-collected samples and the A_2 (marketed) sample was very opposite to these characters, showing their structural and other differences. Also, the color of the original *Mocharasa* is dark blackish brown, but the A_2 (marketed sample) and A_3 was found to be reddish brown and shiny, which is a different character. A_1 is identical to original plant collected *Mocharasa* but A_2 and A_3 are entirely different from A_1 but identical to each other [figure 1a-c and 2].

(2) Physicochemical and phytochemical tests:

The tests such as extractive values suggested that water-soluble contents were more in the A_3 sample (Moringa gum) and A_2 (marketed) sample and that alcohol-soluble extractives were less in these samples and opposite in the A_1 sample, showing their difference in the constituent's solubility in water and alcohol. This may be due to the difference in chemical constituents of all the three samples. Ash values were not very significant, and were nearly similar in all the three samples. Tannin was present in all the samples, which shows that all the three are gummy exudates but quantitative estimation of tannin has to be done.

Gas chromatography showed that some similar peaks may be due to the presence of similar constituents like tannin, but one peak was more in the A_2 sample, which may be due to some adulteration or contamination.

(3) Powder microscopy:

Powder microscopy showed similarity in the presence of xylem fibers in Moringa gum and the marketed sample, which was absent in the authenticated sample. Mucilage was present in all the three samples, which is a common character of gum or exudates.

According to the new standards established for standard crude drugs, standards official crude drug study should include all physicochemical descriptions and also study of adulterants or substitutes. During standardization of drug, study of adulterants for their easy identification and separation is essential. Therefore, accordingly, this study was conducted at a preliminary level.

The A_2 sample was found to be very different in properties to the A_1 sample.

Some of these tests, like appearance, organoleptic characters, alcohol- and water-soluble extractive values, powder microscopy, swelling index, etc. suggested that the A_1 authentic sample was very different and that the A_2 and A_3 samples were very similar to each other. It may be probable that the A_2 sample is actually Moringa oleifera gum but sold as *Mocharasa*.

But, further confirmatory tests and detailed study, such as DNA printing and other higher tests, are required. These are only some of the important tests to identify a difference between the original and the market samples. These are preliminary studies conducted to study the difference.

Conclusion

Mocharasa samples collected from various parts of India showed wide variation in appearance, color, weight, structure, and transparency. In the present study, considering these variations, various samples were selected, and, out of these, the following three were taken: (1) authentic sample A_1, (2) marketed unauthenticated sample A_2, and (3) Moringa gum A_3, to compare within these three samples by various phytochemical and physicochemical tests. Powder microscopy and gas chromatographic analysis and all other tests proved that the marketed sample i.e. A_2 was similar to A_3 Moringa gum sample used as substitute or adulterant to *Mocharasa.*

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