Study of Market Samples of *Khulanjan* for Their Quality Standards

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Abstract

Adulteration and substitution are common in commercial samples of many drugs due to resemblance between two or more drugs which are easily mixed with one another giving confounding characters. Consequently, market samples of some of the drugs are available as either completely substituted or a mixed bag of genuine and substituted drugs. In view of regular use of *Khulanjan* in Unani Medicine and reports of its being admixed with spurious or low quality substitutes, present study was undertaken to study its three samples, two collected from market and one obtained from natural habitat. Pharmacognostical parameters were applied to all the samples to ascertain authenticity of commercially available samples by comparing them with the standard sample. The study consisted of macroscopic, physicochemical and phytochemical studies and spectrophotometery of all the samples. Findings of the study in most of the parameters were found almost similar indicating market samples of the drug to be genuine.

Key words: Unani Medicine, Crude drugs, Market samples, Adulteration, Standardization; *Khulanjan*

Introduction

Centuries old practice of Unani medicine is testimony to its therapeutic potential. It uses drugs of natural sources preferably that of plant origin. Traditional medical systems including Unani medicine that use natural drugs are facing serious problems pertaining to the availability of authentic drugs. Commercially available samples of some of plant drugs are frequently found adulterated or substituted. Growing awareness and increased use of herbal drugs have resulted in injudicious exploitation of wild sources of certain drugs which favored adulteration and substitution. Adulteration and substitution are not only creating problems to physicians and researchers but are also compromising the efficacy of a number of important drugs.

Crude herbal drug market has been in the domain of non medical men, who themselves and the workers they employed to collect the drugs have little idea of identity and quality of drugs and remain least bothered for such attributes. Such practices put a question mark on the authenticity of the crude drugs available in the market. It has been observed that many samples of plant drugs procured from different markets do not match with the description given in the literature. And it becomes a matter of great concern when entirely different samples are available in the market in place of a genuine drug (Bonakdar, 2002).

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Pharmacognosy is a reliable tool by which much information about crude drugs can be obtained (Soni, 2011). Detailed pharmacognostical evaluation gives valuable information about characteristics of crude drugs. If pharmacognostical techniques and classical approaches are applied together for authentication of a drug, better results can be obtained. Regulatory authorities are also in favour of such guidelines. Exclusive literature review and market and field surveys can provide additional benefit in this regard.

Kulanjan (*Alpinia galanga* Linn.) is an important drug of Unani medicine used in common practice and included in a number of pharmacopoeal and non-pharmacopoeal formulations on account of being attributed to possess different pharmacological effects (Shetty, 2005; Verma, 2011; Girija 2014; Kabeeruddin, 2007; Saeed, 2007; Khan 2012). However, it is frequently adulterated with other drugs that have simulating physical appearance. It affects the quality and thereby the efficacy of the preparations. In view of the above, three different samples of *Khulanjan* were taken up for the present study. One sample was collected from the habitat and was considered as standard. Two samples of the same drug were obtained from two different markets. All three samples were studied on certain Pharmacognostical parameters with an aim to compare the findings with one another to observe the differences, if any, between the samples.

Materials and Methods

Materials

Two market samples under the name of *Khulanjan* were procured from Bengaluru and Hyderabad's herbal drug markets and were designated as A and B, respectively. These samples were kept unidentified. The third samples named as C was collected from natural habitat (Herbal garden of National Institute of Unani Medicine (NIUM), Bengaluru (Figure 1) and was identified as *Alpinia galanga* Linn, by S. Noorunnisa Begum, Senior Asst. Professor, FRLHT, Bengaluru vide authentication certificate no. 3832. Sample C served as the standard sample. Voucher specimens of all the samples have been deposited in the drug museum of NIUM, Bengaluru. All the chemicals and reagents used in this study were of analytical grade.

Methods

Macroscopic/Organoleptic studies

The organoleptic characters like color, odor, taste, shape, size, and surface of all the samples were examined by naked eye as described by Wallis (2005).

Physicochemical studies

Total ash, acid insoluble ash and water soluble ash were determined by the method described in Physicochemical standards of Unani Formulations (Anonymous, 1987); extractive values in petroleum ether, benzene, chloroform, acetone, ethanol, and distilled water were determined by the method described in British pharmacopoeia (Anonymous, 1968). Moisture content was determined by the loss on drying method (Khandelwal, 2008). The pH value of 1% and 10% aqueous solution was checked by the method described in Physicochemical Standards of Unani Formulations (Anonymous, 1987).

Phytochemical studies

For preliminary phytochemical studies of extracts taken in different solvents *viz*. Petroleum ether, benzene, chloroform, acetone, ethanol, and distilled water were subjected to various qualitative phytochemical tests such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, phytosterols, fixed oils, coumarins, diterpenes, flavonoides proteins and amino acids etc by different methods (Anonymous, 1987; Brewster, 1971; Bhattacharjee and Das, 1969; Khandelwal, 2008; Pandey, 2013).

Spectrophotometery (Spectrum scanning)

Spectrophotometery was performed with the help of UV-Vis Spectrophotometer (model Lab India 3000). Extracts of test drugs were analyzed against blank sample for visible wave length range (360-190 nm). The parameters were set and dark current correction was performed to ensure the accuracy of the measurement. Baseline correction was performed with the sample control cell, and then sample of drug was analyzed. Peak picking was done by threshold value. The observations were saved in graphical as well as tabular form to note maximum absorbance against particular wave length and the number of peaks. Other specifications included Spectral Bandwidth, 2.00 nm; Spectrum Performance: Scan Range, 190.00-900.00; Measure Mode, Abs; Interval, 5.00 nm. Speed: Fast.

A B C

Figure 1: Various samples of Khulanjan

Table 1: Organoleptic characters of various samples of *Khulanjan*

S. No.	Characteristics	Sample A	Sample B	Sample C
1.	Shape	Cylindrical, branched	Cylindrical, branched	Cylindrical, branched
2.	Size	2-4cm long, 0.1-0.2cm diameter	2-5cm long,0.1-0.2cm diameter	3-8cm long, 0.1cm diameter
3.	Colour	Dark brown	Reddish brown	Yellowish brown
4.	Odour	Pungent & Aromatic	Pungent & Aromatic	Pleasant & Aromatic
5.	Taste	Spicy	Spicy	Spicy & Sweet
6.	Surface	Rough	Rough	Rough

Table 2: Ash values of of various samples of *Khulanjan*

Samples	Ash Values		
	Total ash	Acid insoluble ash	Water soluble ash
A	4.25±0.03	0.89±0.07	2.75±0.16
B	4.91±0.04	3.76±0.27	1.59±0.33
C	5.64±0.15	2.80±0.19	1.45±0.09

Table 3: Extractive values of various samples of *Khulanjan* in different solvents

Samples	Solvents					
	Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Aqueous
A	2.56±0.30	1.82±0.38	1.744±1.744	3.53±0.392	2.10±0.20	11.05±0.38
B	1.14±0.26	1.45±0.30	0.307±0.307	1.51±0.34	2.10±0.20	23.94±1.07
C	2.26±0.25	0.55±0.09	0.342±0.342	1.84±0.29	10.83±0.46	16.05±0.52

Table 4: pH, Moisture Content and Solubility of various samples of *Khulanjan*

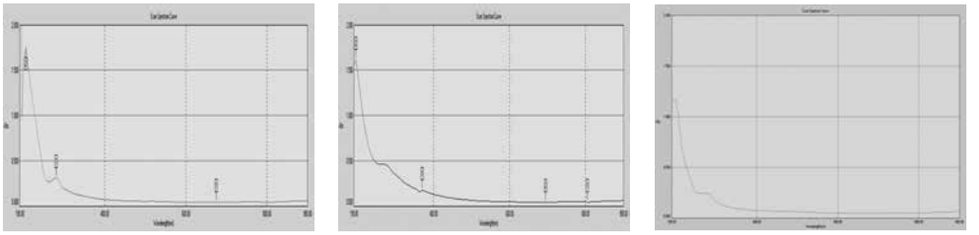
	Samples					
	1% solution			10% solution		
	A	B	C	A	B	C
pH	4.47±0.38	4.98±0.21	4.74±0.36	4.74±0.9	5.46±0.9	4.65±0.10
Moisture Content	10.57±0.04	9.79±0.08	9.13±0.01	-	-	-
Solubility	17.76±1.16	15.78±0.2	20.97±0.5	-	-	-

Table 5: Fluorescence analysis of powders of various samples of *Khulanjan*

S. No.	Treatment	Fluorescence					
		In daylight			In UV light		
		A	B	C	A	B	C
1.	Powder as such	Light brown	Light yellow	Yellowish brown	Gray	Gray	Gray
2.	Powder + 1N HCL	Red	Black	Reddish brown	Black	Greenish black	Dark brown
3.	Powder + 1N NaOH	Black	Reddish Yellow	Brown	Greenish Black	Greenish Black	Dark brown
4.	Powder + 50% HCL	Red	Red	Light Yellow	Greenish brown	Greenish	Dark Gray
5.	Powder + 50% H_2SO_4	Reddish brown	Gray	Reddish brown	Greenish black	Black	Dark green
6.	Powder + 50% HNO_3	Red	Red	Red	Black	Black	Brown
7.	Powder + Methanol	Red	Yellowish	Yellow	Gray	Gray	Gray
8.	Powder + Methanol + 1N NaOH	Reddish brown	Yellowish brown	Yellowish brown	Black	Yellowish brown	Dark brown

Table 6: Spectrophotometery: Aqueous extract

Aqueous extract								
Sample A			Sample B			Sample C		
Peak	Wave length (nm)	Absorbance	Peak	Wave length (nm)	Absorbance	Peak	Wave length (nm)	Absorbance
Peak-1	280.00	0.314	Peak-1	70.00	370.00	Peak-1	745.00	0.053
Peak-2	205.00	0.133	Peak-2	195.00	195.00	Peak-2	275.00	0.248

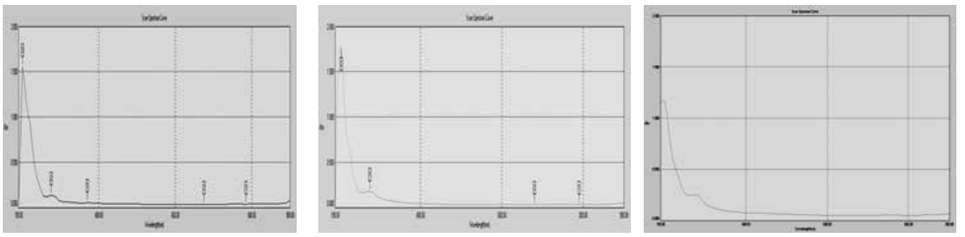


A B C

Figure 2: Spectrum scan curves: Aqueous extract

Table 7: Spectrophotometery: Ethanol extract

Ethanol extract								
Sample A			Sample B			Sample C		
Peak	Wave length (nm)	Absorbance	Peak	Wave length (nm)	Absorbance	Peak	Wave length (nm)	Absorbance
Peak-1	370.00	0.053	Peak-1	275.00	0.179	Peak-1	275.00	0.062
Peak-2	275.00	0.133	Peak-2	205.00	.779	Peak-2	195.00	1.535
Peak-3	200.00	1.558	-	-	-	-	-	-



A B C

Figure 3: Spectrum scan curves: Ethanol extract

Results and Discussion

A number of studies on commercial samples of plant origin drugs have revealed that many drugs were substituted and/or adulterated (Ansari, 1994; Afaq, 1994). Over the decades, on account of growing awareness about herbal drugs and the increasing demand for herbal products for various therapeutic application mindless exploitation of wild sources of these drugs has become rampant. Many traders of herbal drugs have indulged in malpractices taking advantage of this situation (Ansari, 1994; Afaq, 1994). Similar looking drugs are easily mixed and therefore the market samples of many crude drugs are either totally substituted or marketed as a mixture of genuine and substituted drugs. Therefore, it is necessary to standardize all the single drugs to rule out misidentification, adulteration and substitution of herbal drugs.

Standardization is the corner stone for ensuring the authenticity and genuineness of herbal drugs. Strategies have been made for standardization by most of the regulatory authorities which are based on macroscopy, microscopy, physicochemical and analytical studies.

Morphological characteristics are important in describing the deterioration of drugs due to faulty harvesting, shipment and storage. In our study we applied macroscopy to all the samples. The findings showed similarity with minor differences in all the samples with respect to shape, size and surface, however the colour was found a bit different (Figure1 a, b, c and Table 1). This may have arisen because of different storage conditions, age and source of the drugs.

Physical parameters for organized drugs usually includes ash values, extractive values, moisture content, solubility, and pH. We applied all these parameters. The findings of all three samples (A, B and C) were found to have similar character with minor or negligible differences, which were within the normal range. Acid insoluble ash of sample B was higher when compared with C; high acid insoluble ash indicates contamination with earthy material (Evans, 2008) (Table 2). Extractive values in a particular solvent are indicators of originality of the drugs. The petroleum ether extractive value of A and C was similar. Less value of sample B may be because of excessive drying or oldness of the drug. The benzene extract of A and B was found to be more than C. The chloroform extractive value of A was more than C. The acetone extractive values of sample A were more than C, whereas that of B was similar to C. The extractive value of C in ethanol was more than A and B. The aqueous extract of sample B was estimated to be more than C. but that of sample A was less than standard sample C (Table 3). The factors which influence the quality of an extract depends upon the extraction procedure, type of extraction, time of extraction, temperature, nature of solvent and polarity etc. (Tiwari *et al*., 2011). Slight differences in the extractive values may be taken as normal in view of the above factors. Aqueous solubility was found within the normal range. Moisture content of sample A and B was nearly equal (Table 4). The pH values of the three samples in 1% and 10% aqueous solution were also found within the normal range (Table 4). Preliminary phytochemical studies are not only good indicator of genuineness of drug but are considered more reliable than the physical parameters. *In certain cases, a particular test on a drug shows negative results but at the same time it shows positive result in other tests. In our study, it happened very often*. Preliminary phytochemical test carried out on the extract prepared in various solvents, revealed presence of alkaloids, carbohydrates, glycosides, terpenes/phytosterols, fixed oil, flavonoides, diterpenes, quinones, anthraquinones, saponins, proteins, amino acids and coumarins in all the samples. Terpenoids, which contains volatile oils, is insoluble in water and soluble in organic compounds (Doughari, 2012; Ahmad, 2007). Phenols and poly phenols can be extracted in water, ethanol, methanol, and acetone extracts (Tiwari *et al*., 2011). Fixed oils were positive in all the three samples of petroleum ether extract. Flavonoides can be extracted through, ethanol, methanol, chloroform, ether, and acetone (Tiwari *et al*., 2011). Tannins are soluble in water, dilute alkali, alcohol, and glycerol (Doughari, 2012;

Ahmad 2007). Saponin is soluble in water and alcohol. It is insoluble in non-polar organic solvents like benzene, hexane, chloroform etc. (Doughari , 2012; Ahmad, 2007). Proteins and amino acids are soluble, dilute in water, dilute acids, dilute alkali solutions, and dilute salt solutions, 70% alcohol. Xanthoproteinic test is used for the detection of presence of aromatic ring in amino acids (Doughari, 2012; Ahmad, 2007). Tests for inorganic constituents showed positive results for sulphates, iron and chloride in all the three samples.

Fluorescence analysis was also carried out during the study. Findings of our study revealed minor differences among the samples (Table 5) however the differences were not found to be significant.

Spectrophotometery is the measurement and interpretation of electromagnetic radiation absorbed or emitted (Sankar, 2010). This highly sensitive technique is frequently used for differentiation between similar looking drugs with the help of peaks observed in respect of different samples. If, it is allowed to run against a standard marker, phytoconstituents may also be characterized. But, in our study, spectrophotometery was appropriated to run against the blank sample, therefore the peaks could not be interpreted, however the nature and number of peaks can give a rough idea about the difference or similarity between two or more drugs. In our study, aqueous extract of all the samples gave two peaks but with minutely different wave lengths and absorbance. But, the ethanol extract gave three peaks in sample A and two peaks each in sample B and C again with minutely different wave lengths and absorbance (Table 6, 7 and Figure 2, 3). The findings suggested that there is inconsequential difference among the three samples. It indicates that samples available in the Hyderabad and Bangaluru are reliable and can be used for te preparation of herbal products. However the difference in the number of peaks in sample A must be investigated further to know whether it is an indicator of a phytoconstituent or something extraneous.

Conclusion

The study revealed similarity among the three samples collected from Bengaluru, Hyderabad and from the habitat of NIUM, Bengaluru. The findings of the study demonstrated that different physicochemical and analytical characters of market samples were same as that of the standard one, therefore, it was concluded that the market samples of *Khulanjan* collected from the markets were genuine.

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