INNOVARE JOURNAL OF AYURVEDIC SCIENCES

Vol 1, Issue 3 , 2013 ISSN:2321-6824

Research Article

CHARACTERIZATION OF *AFTEEMOON HINDI/AKASHBEL* SOLD IN WHOLESALE MARKET OF HERBAL DRUG AT KHARI BAOLI, DELHI

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Received: 7 September 2013, Revised and Accepted: 18 September 2013

ABSTRACT

Objectives: To question reliability of drugs available at wholesale market of crud herbal drug in Delhi by analyzing physiochemical properties of a drug, *Afteemoon Hindi/ Akashbel.*
Method: Standardization of crude drug is becoming must for drug industries for GMP, clinical practioners and research works for effective and accurate results. Afteemoon Hindi also called Akashbel is important plant used as drug in Unani and Ayurvedic system of medicine with many bioactive molecules. The drug is used singly and also constitutes important part in various pharmacopeial and patented compound drugs. Study was executed in to characterize the properties of this important drug available in market. Parameters were botanical identification, foreign materials, moisture content/loss on drying, ash values, pH and TLC.
Results and conclusion: The parameters show slight variations in results from previously standardized results for the same drug. Therefore it is to conclude that these variations may alter expected outcome of the drug in patient's treatment and need to be work out authentication of drug before purchasing.

Keywords: *Afteemoon*, Ash value, Successive distillation, pH, TLC

INTRODUCTION

Availability of authentic crude herbal drug has been of great concern now a day. Industries for preparation of compound Ayurvedic and Unani drugs are now on increase. Major industries in Delhi and NCR as well as other states of India rely on available drug in wholesale market of herbal drug at Khari Baoli, Delhi. But the drugs available need to be indentified on botanical, physical and chemical parameters for GMP (Good Manufacturing Practices) in drug industries. Clinical efficacy of drug to patients is also altered due to administration of improperly identified drug or adulterated drugs.

Afteemoon Hindi/Akashbel is important drug used in Unani and Ayurvedic system of medicine. There are 20 pharmacopeial drug in Unani System alone, known to me which have *Afteemoon Hindi* as an ingredient including *Majoon Dabidulward, Sharbat Kasoos, Sharbat Dinar, Sharbat Ahmad Shahi, Sharbat Bazoori Haar, Itrifal Afteemoon* are few of them. There may be marked alteration in active bimolecular compound with desired activities if drug available in market is not properly indentified before purchase. It may also arise due to substandard, old, degenerated, decayed or adulterated drug. Researches had listed a number of chemical constituents found in Afteemoon Hindi as shown in the table no.1

Table No. 1: It shows different chemical constituents along with their references

Dulcitol, luleolin, quercetin, lutcolin Cuscutalin, cuscutin, amrabelin(seed), wax	The Unani Pharmacopoeia of India, 2002[1] Chopra R, Nayar S & Chopra I, 1956[2]
Quercetin, Cuscutine, Dulcitol, luteolin, luteolin, Resins	Singh M & Himdari P, 2005[3],Nandkarni, 1989[4]
5-caffeoyl-quinic acid (chlorogenic acid),	Löffle C, Sahm A, Czygan F, Proksch P, & Wary V, [5]
3,5-dicaffeoyl-quinic acid, 4,5-dicaffeoyl-quinic acid,	
O-glycosides quercetin-3-O-ß-galactoside	
Quercetin-3-O-ß-glucoside	
Kaempferol-3-O-ß-galactoside and kaempferol-3-O-ß-glucoside	
7'-(3',4'-dihydroxyphenyl)-N-[(4-methoxyphenyl) ethyl] propenamide	Matsui T, Yoshimoto C, Osajima K, Oki T & Osajima Y: 1996[6]
7'-(4'-hydroxy,3'-methoxyphenyl)-N-[(4-butylphenyl) ethyl]propenamide	Kelkar C & Haraff R H:1984[7]
6,7-dimethoxy-2H-1-benzopyran-2-one	Theodore C: 1908[8]
3-(3,4-dihydroxyphenyl) -2-propen-1-ethanoate	Shastri B:1962[9]
6,7,8-trimethoxy-2H-1-benzopyran-2-one	Anis E, Ullah N, Mustafa G, Malik A, Afza N & Bader Y:1999[10]
3-(4-O-beta-D-glucopyranoside-3,5-dimethoxyphenyl)-2-propen-1-ol	Kelley C, Sahm A, Czygan F, Proksch P & Wary V: 1976[11]
2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-O-beta-D-glucopyranoside-4H-1-benzopyrane-4-one	Chemesova I: 1990[12]
reflexin, 5-hydroxy-7-methoxy-6-(2,3-epoxy-3-methylbutyl)-flavanone	(Tripathi V, Yadav S & Upadhyay A :2005[13]
swarnalin and cis-swarnalin,coumarin, 5,6,7-trimethoxycoumarin	Uddin S, Shilpi JA, M M: 2007[14]
Inorganic: Aluminum, iron, calcium, sodium ,potassium	CCRUM:[15]

Table No.1 Chemical constituents of Afteemoon Hindi

Standardization as defined in the text for guidance on the quality of herbal medicinal products means adjusting the herbal drug preparation to a defined content of a constituent or group of substances with known therapeutic activity. [16]

Standardization of available *Afteemoon Hindi* in market of Khari Baoli was carried out with references to foreign materials, moisture content/loss on drying, ash value, pH and TLC after biological identification

METHODS AND OBSERVATIONS

Biological Identification

Afteemoon is obtained from Khari Baoli, wholesale market of herbs in Delhi. *Afteemoon* needs its botanical identification as there are number of species of Cuscuta having almost same morphological character, important among them is Cuscuta chinensis called *Afteemoon Villayti* in Unani medicine. A sample of *Afteemoon* (whole plant) was given to Department of Botany, Jamia Hamdard for its identification. Identification was done with respect to its morphological characters and drug was found to be authentic and identified as Cuscuta reflexa. Thus, the whole classification of the drug can be mentioned as follows according to United States plant profile of Natural resources conservation services.[17]

Table No. 2: It shows taxonomy of Afteemoon Hindi

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Convolvulaceae
Genus	Cuscuta
Species	*Cuscuta reflexa* Roxb.

Foreign Matters

This parameter is to find the percentage of foreign materials present in the test drug.

A container made of paper was weighed and some amount of *Afteemoon* was put into it and weighed again. The drug was spread on white paper sheet and foreign matters were identified, picked and removed with best possible efforts. The rest of the drug was again weighed with paper container. The result was evaluated in percentage. The results and calculations are as follows:

Weight of paper container (W1) – 42 g.
Weight of drug + Paper container (W2) – 117 g.
Weight of selected drug + Paper Container (W3) – 114 g
Weight of total drug taken (W4) = (W2 – W1) = (117 – 42) = 75 g.
Weight of selected drug (W5) = (W3- W1) = (114 – 42) = 72 g
Weight of Foreign Matters (W6) = (W4 – W5) = (75 – 72) = 3 g.
Thus, Percentage of foreign matters = W6/W4 X 100 = 4%

Result: Foreign matters in the drug is 4%.

Loss on drying / Moisture content:

This parameter is used to determine the amount of moisture present in the sample. The crude drug sample 5g was placed on a dry Petri dish. The Petri dish along with drug was dried at 105 ^{o}C for 2 hours in oven and weighed. The drying was continued until two successive reading matched each other.

Calculations and results are as follows:

Weight of dry Petri dish, P1 = 50.4 g.
Weight of Petri dish + drug, P2 = 55.4 g.
Weight of Petri dish + drug after drying for 2 hrs, P3 = 55.15 g.
Weight of Petri dish + drug after drying for 1 hrs again, P4 = 55.00 g.
Weight of Petri dish + drug after drying for 1 hrs again, P5 = 55.00 g.
Weight of drug W1 = (P2 – P1) = (55.4 – 50.4) = 5g
Weight of properly dried drug W2 = (P5 – P1) = (55.00 – 50.4) = 4.6 g.
Loss on drying (in Grams) = (W1 – W2) = (5.0 – 4.6) = 0.4g
Loss on drying (in percent) = (0.4/5 x 100) = 8%

Result: Loss on drying/ moisture content in the sample was found 8%.

Ash Value

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drug results in ash residue consisting of inorganic material (metallic salts and silica). This value varies within fairly wide limits and is, therefore, an important parameter for the purpose of evaluation of crude drugs. The ash value determined is the total ash, the acid insoluble ash and the water-soluble ash.

Total Ash

Crucible is weighed (C1) and then 5g of dry drug is kept in it and weighed again (C1 + D). Crucible with drug was kept in Muffle furnace at not more than 450 degree centigrade for 4 hrs. Crucible with resulting ash was cooled and weighed. Now, again crucible with ash was kept in Muffle furnace for two hours at same temperature and weighed again. The weight was found constant (C1 + A). The process was repeated for tree times and all the values were noted as represented below in table form.

Table No. 3: It shows different readings in calculation of Total Ash.

Wt. in g ->	**C1**	**C1 + D**	**C1 + A**	**Total Ash**	**Ash %**
I	38.26	42.26	38.57	0.31	6.2%
II	32.06	37.06	32.48	0.42	8.4%
III	38.26	42.26	38.55	0.29	5.8%

Table No. 3

Thus, Average Total ash (in %) = (6.2 + 8.4 + 5.8)/3 =6.8%

Result: Total Ash == 6.8%

Water Soluble Ash

Total Ash was calculated by the above mentioned method and 20 ml of water was put in the crucible and heated over waterbath. The resultant was filtered through ash-less filter paper and again the crucible was kept in Muffle furnace for 2 hours at 450^{o}C. Crucible was cooled and weighed again.

Calculations and results are as follows;

Weight of the crucible = C1 = 38.26 g
Weight of the crucible + Drug = C1+D = 42.26 g
Weight of the crucible + Ash = C1+A = 38.57 g
Total Ash = 38.57 – 38.26 = 0.31 g
Weight of crucible + Insoluble Ash = C1+A' = 38.41g
Total insoluble ash = 38.41 – 38.26 = 0.15
Total soluble ash = 0.31 – 0.15 =0.16
Thus, Water Insoluble Ash % = (0.16/5 X100) = 3.2%
Result: water Soluble Ash percent = 3.2%

Acid Insoluble Ash

Total ash was calculated as mentioned. The total ash was boiled with 25 ml of 2N HCl for 5 min. The insoluble matter was collected on an ash less filter paper after filtering. The crucible with ash less filter paper and insoluble matters was again kept in Muffle furnace for 2 hrs at same temperature. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.

Calculations and result are as follows:

Weight of the crucible = C1 = 32.06 g
Weight of the crucible + Drug = C1+D = 37.06 g
Weight of the crucible + Ash = C1+A = 32.48 g
Total Ash = 32.48 – 32.06 = 0.42 g
Weight of crucible + Acid Insoluble Ash = C1+A' = 32.15g
Total Acid insoluble ash = 32.15 – 32.06 = 0.09 g
Thus, Acid Insoluble Ash % = (0.09/5 X100) = 1.8%
Result: Acid Insoluble Ash = 1.8%

Sulphated Ash

To the sample of 5g drug, 10ml of 25% Sulphuric acid is added in a weighed crucible. Muffle furnace was set at 600^{o}C.and the crucible was kept in it for 5 hours. Crucible was cooled and weighed. It was again kept in furnace for 2hr and constant weight was found.

Calculation and results are as follows:

Weight of the crucible, C1 = 38.26 g
Weight of the crucible + Drug = C1+D = 42.26 g
Weight of crucible + Sulphated ash = 38. 58 g
Therefore, Total Ash = 38.58 – 38.26 = 0.32 g

Thus, percentage of Sulphated ash = (0.32/5 X100) = 6.4%
Result: Percentage of Sulphated ash is 6.4%

pH value

Whole plant of *Afteemoon* is taken, dried and powdered finely. 2 gram is taken in 10 ml distilled water and dissolved with the help of sonicator and 20% aq. solution (w/v) was made. Reading was taken through pH meter.

Result; pH of powdered Afteemoon is 7.5

Successive Extraction:

The dried and coarsely powdered material (25 gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, methanol and water. The extracts were evaporated to dryness and their constant weights were recorded.

Table No. 4: It shows dried extract value of the test drug in different solvents.

Wt. in Gram→ Solvent↓	Wt.of Beaker(B)	Wt. of beaker + extract	Wt. of extract	Percentage
Petroleum ether	95.71	96.29	0.58	2.32%
Chloroform	95.32	95.81	0.49	1.96%
Methanol	96.--	103.54	5.35	21.4%
Water	102.10	107.86	5.76	23.04%

Table: 4

TLC (Thin Layer Chromatography)

It is a method or procedure for the separation and identification of mixture of compounds. Here, as a single herb has a number of active compounds therefore TLC of each extract in different solvent system was tried and best among them are represented in the table.

Petroleum Ether extract

Solvent System---- Petroleum: Ethyl acetate = 8:2

Table No. 5: It shows *Rf* Values in TLC of Petroleum Ether Extract of test drug in different sprayl treatment in above mentioned solvent solution.

R = 54 mm

View/ sprayl treatment ↓	Number of spots and position in mm	*Rf.* Values
Naked	(6)-5,18,24,28, 32,40	0.09,0.33,0.44,0.52,0.59,0.74
UV Chamber	(5)-5,24,28,32, 40	0.09,0.44,0.52,0.59,0.74
Iodine Vapour	(6)-5,24,28,40, 45,50	0.09,0.44,0.52,0.74,0.83,0.92
Suphuric acid spray	(7)-5,24,28,32,40,45, 50	0.09,0.44,0.52,0.59,0.74,0.83,0.92

Table no. 5

Solvent System---- Petroleum:Toluene:Ethyl acetate = 8:1:1

Table No. 6: It shows *Rf* Values in TLC of Petroleum Ether Extract of test drug in different sprayl treatment in above mentioned solvent solution.

R = 57 mm

View/ sprayl treatment↓	Number of spots and position in mm	*Rf.* Values
Naked	(3)-6,12,30	0.10,0.21,0.52
UV Chamber	(4)- 6,12,24,30	0.10,0.21,0.42,0.52
Iodine Vapour	(6)-6,12,16,21, 37,50	0.10,0.21,0.28,0.37, 0.65,0.87
Suphuric acid spray	(3)-6,12,37	0.10,0.21,0.65

Table no. 6

Chloroform Extract

Solvent system –Chloroform: Methanol = 9:1

Table no. 7: It shows *Rf* Values in TLC of Chloroform Extract of test drug in different sprayl treatment in above mentioned solvent solution

R= 55mm

View/ sprayl treatment↓	Number of spots and position in mm	*Rf.* Values
Naked	(3)-5,10, 40	0.09,0.18,0.72
UV Chamber	(5)- 5,10,23,36,43	0.09,0.18,0.41,0.65,0.78
Iodine Vapour	(4)-9,16,23,36	0.16,0.29,0.41,0.65
Suphuric acid spray	(4)-9,16,23,36	0.16,0.29,0.41,0.65

Table No. 7

Methanolic Extract

Solvent system – Chloroform: Methanol = 8:2

Table No. 8: It shows Rf Values in TLC of methanolic Extract of test drug in different sprayl treatment in above mentioned solvent solution.

R= 52mm

View/ sprayl treatment↓	Number of spots and position in mm	*Rf.* Values
Naked	(3)-29,34, 47	0.55,0.65,0.90
UV Chamber	(5)-15,29,31,34,45,48	0.29,0.55,0.59,0.65,0.86,0.92
Iodine Vapour	(5)-10,15,29,35,45	0.19,0.55,0.59,0.67,0.86
Suphuric acid spray	(4)-20,29,35,46	0.38,0.59,0.67,0.88

Table no. 8

Fig. No. 1: It shows spots obtained in TLC plate for Petroleum ether extract of *Afteemoon Hindi* in solvent solution-Petroleum: Ethyl Acetate=8:2

Fig . No.2: It shows spots obtained in TLC plate for Methanolic extract of Afteemoon in solvent solution-chloroform: methanol=8:2

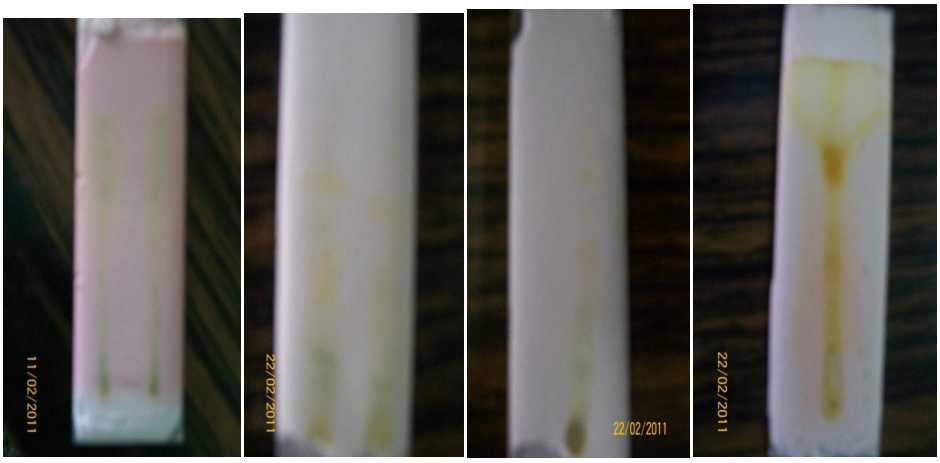

Fig. No.3: It shows different trials made on TLC plates for *Afteemoon Hindi* in different solvent solution

RESULTS AND DISCUSSION

Botanical identification, however was done through institutional department of Botany, was considered authentic. As the drug was dry, no cross-section microscopic view of any part of plant can be carried out but seeds present were verified and the drug was matched with previously preserved *Afteemoon Hindi* and differentiated from its adulterant *Afteemoon villayti* by naked eye. Results obtained from Botany department states "Identified as Cuscuta reflexa *Roxb*. with some foreign matters"

Foreign materials estimation was done through hand picking. It was found to be 4% of total drug weight. According to CCRUM, foreign organic matter found in sample of Afteemoon Hindi was found to be 2.3%. This might be duo to variation in host plant.[18]

Result for loss in weight shows 8% in w/w.

Results of Total ash value, water soluble ash, acid insoluble ash and sulphated ash are 6.8%, 3.2%, 1.8% and 6.4% respectively. The study by CCRUM shows Total ash value as 9.97%, Water soluble ash value 4.92% and acid insoluble ash as 1.62% [18]. Mild variations had been noticed in the result.

pH value of the test drug 20% aq. Solution (w/v) was found to be 7.4 while pH value mention in book of CCRUM is 6.55% for 1% solution and 6.05% for 10% solution.[18]

On successive extraction with petroleum ether, chloroform, methanol and water the weight percent on drying the beaker was found to be 2.32%, 1.96% , 21.4% and 23.04% respectively. Researchers have shown the results of successive extraction for petroleum ether is 2.80%, for Chloroform is 2.95%, ethanol as 7.56% and distilled water is 19.88%.[18] Variations are also noted here.

The same extracts were used for Thin Layer Chromatography and viewed firstly through naked eyes and then in UV Chamber and Iodine chamber and also with sulphuric acid spray. The results are shown in table no. 4, 5, 6 and 7 and fig no. 1,2 and 3.

TLC by CCRUM was executed in solvent solution of one part of diethyl ether and four part of petroleum ether. And sproyl treatment of iodine vapours. Six spots were seen in the extract of Petroleum ether with Rf, Values 0.25, 0.35, 0.45, 0.55, 0.85, and 0.95. Extract from chloroform shows two spots with Rf. Values 0.25 and 0.35. Acetone extract shows only one spot with Rf. Value 0.25[18]

CONCLUSION

It is to say that there is wide range of drugs available in the market with the same name; however they differ greatly due to adulteration, close variety or improper identification. In spite of being identified as *Afteemoon Hindi (*Cuscuta reflexa *Roxb.),*physiochemical analysis had shown variations. Thus it is recommended for herbal drug industries to undergo identification of drug with physical and chemical parameters for each batch of production. Physicians should also workout ways to indentify exact drug and does not rely on market so that expected results can be obtained.

ACKNOWLEDGMENT

The work presented beside having effective contribution by co-authors, cannot undermine the contribution of others. Prof Shakir Jameel (Presently Director General of CCRUM) had inspirational attitude which helped us a lot. Dr Mushtaq, Dr Umar Jahangeer, Dr Nafees Iqbal, Dr Abdul Nasir, Dr Qutubuddin and Dr Naushad Rana are exceptionally need to be mentioned. Thanks to Head, Dept. of Botany for identifying the drug for us and also to management of Majeedia hospital and Jamia Hamdard for providing ample environment for research work to be carried out. List of helping hands

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