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New technologies and interventions for the standardization of Unani drugs

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Abstract
"Quality'' word is taken from latin which means "of what kind" and covers content and properties of an object. Unani Drugs quality is very important as safety of alternative medicinal plants has been questioned due to its side effects. In order to maintain the quality and efficacy of these drugs it is important to standardize them to keep away from adulterated medicines in the market.
Some important issues related to Unani's quality have been addressed in this article, and a series of physiochemical parameters has been discussed to monitor the quality of a Unani poly-herbal formulation and to assess the quality of medicinal plant materials. We have also made an attempt to discuss the regulatory laws dealing with standardization and the need for their implementation as well as improvisions. Based on the literature reviewed, in our opinion three areas need immediate attention for strengthening Unani systems of medicine in India that are sharing of benefits, industrial investment and standardization of Unani medicines.

Keywords: HPTLC, TLC, LC-MS, HPLC, ^{1}H-NMR, AFs, R_f

Introduction
85-90% of World's population relies on Unani medicine according to World Health Organization [1]. Apart from its increasing demand it has arisen many issues regarding undesirable health effects due to variable quality, efficacy, and contents of Unani products and therefore it should meet the requirements of quality, safety and efficacy [2-4]. Most of the Unani drugs are found adulterated with toxic compounds, contaminated with pathogenic microbes or natural toxins like aflatoxins (AFs) [5-7]. Several guidelines have been laid regarding standardization of raw Unani products by WHO [8-12].

Remedy for the problem
Plant materials are used as home remedies, as drug products, and as raw material for the pharmaceutical industry in developed as well as developing countries moreover they signify a significant proportion of the total drug industry. As far as medicinal plants and Unani products are concerned efficacy, quality and safety is a key issue that needs to be answered. It is clear for sure that the Unani industry will have a promising future in India when linked with drug market, industries and scientists. For the better understanding of the applications of Unani medicines quality control data on safety and efficacy and standardization of methods are required. Therefore, it is the need of the hour to set up internationally recognized guiding principle for assessment of their quality. It is evident that the dependency on Unani drugs should be decreased and the switch over to health care friendly Unani products should be increased. Yet, ameticulous search for medicinal plants is needed as it is not an easy task, appropriate strategy for their identification, confirmation of the technical methods of isolation of active ingredients, preclinical estimate of their pharmacological and toxicological profiles, and clinical evidence of their importance. Clinical trials should be done in order to know the average effective dose for any drug, as well as future side effects a Unani compound may cause. In a nutshell, analysis of both Unani and synthetic drug should be same that is with controlled clinical trials. This will not only improve the quality of the drug and also encourage the practitioners to be more engaged in the standardization process.

WHO Guidelines for Standardization of Unani Formulations
Following fundamental parameters are laid by WHO to Standardized the quality control parameters for Unani formulations:

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1. To check the quality of plant preparations, crude drugs material and finished products.
2. To assess the stability and shelf life.
3. Documentation of safety; Safety assessment based on experience or toxicological studies.
4. Evaluation of biological activity and of efficacy Assessment by ethnomedical information.

Organizational Setup in India for the Regulation of Unani Drugs

This is the prime responsibility of the governing authorities to ensure that consumers get the best medication, which assure purity, safety, potency, and efficacy. Their acceptability in modern system of medicine requires quality control of crude drugs and Unani formulations. In this regard the major problem faced by the Unani drug industry is their formulations and non-availability of strict quality control report for Unani materials [13]. Below is the flow chart given by the organizational setup in India for regulations of Unani drugs at central and state level [14]. Figure 1
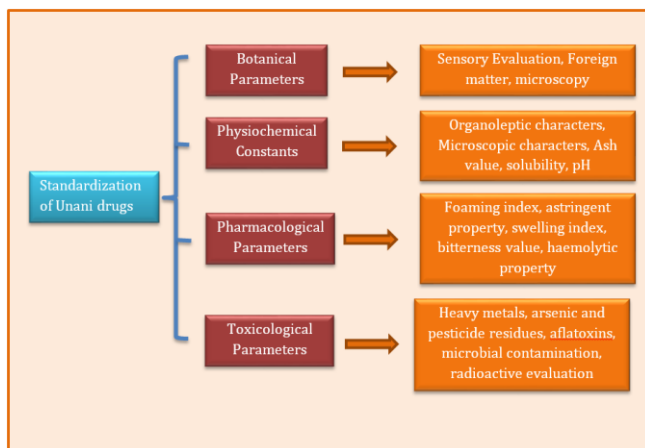

Fig 1: Standardization of Unani Medicine.

The figure shows standardization of Unani medicines the following being the parameters – Botanical parameters, physicochemical, pharmacological and toxicological parameters.

Regulatory laws in India for Unani drugs

Some of the regulatory laws in India for the regulation of Unani drugs are: Drugs and Cosmetics Act, 1940, Indian Medicine Central Council Act, 1970, Drugs and Magic Remedies Act (Objectionable Act), 1954, Biological Diversity Act, 2002, Wild Life Protection Act, 1972, and Indian Forests Act, 1865 [15].

Comparison of Validation Models of Unani Drugs vs Synthetic drugs

We must amalgamate newer techniques in order to regulate the quality of Unani drugs in better way and terms to higher degree. According to USFDA validation is defined as documented evidence which gives a high degree of promise that a specific process will constantly produce a product meeting its predefined standards and quality attributes. Previously, it was misunderstood that validation is nothing but wastage of time and wealth. But with the passage of time this notion is get changed i.e. validation is not wastage of time and wealth but it is an investment of time and

money which results in the production of a worth product. The concept of validation is for synthetic drugs but its use to Unani drugs is limited. There is no such regulation to look for validation except WHO who look after the validation of Unani drugs but again the issue is that WHO also focuses on very small piece of validation. This is the reason the authors want to emphasize on the validation model and the idea of validation and also for manufacturing of Unani drugs. The mostly used validation model for manufacturing of synthetic drugs is shown in Figure 2.1 and Figure 2.2

Fig 2.1: Validation model for Synthetic drugs.

The figure shows the validation model in case of Synthetic drugs. 'Input' here means vendor certification. Then the product is processed and finally certified.

Fig 2.2: Validation model for Unani drugs.

The figure shows the validation model in case of Unani drugs. 'Input' here means vendor certification as well as manufacturer's certification and the product is processed to be certified in the market.

The same validation model is applicable for both Unani drugs and synthetic drugs except there are some limitations like in case of synthetic drugs only vendor certification (Input) is needed but with Unani drugs the situation is changed now both vendor certification as well as manufacturer's certification is needed . The manufacturer certification is necessary but it is difficult to get one in today's Unani industry. Certification of manufacturer is done for analyzing the strength of Unani.

New Methods or Technologies for Standardization

Often TSM falls into due to lack of standardization of Unani extracts it results in variability in efficacy and this most often leads to disrepute to TSM. This is due to the fact that plant preparations are chemically complex and contain compounds that may act together to produce synergism. The composition of these compounds in a plant may vary due to environmental factors making it necessary to standardize the crude extract [16]. Standardization has been traditionally carried out either by bioactivity guided fractionation or by obtaining chemical FP through HPTLC/HPLC.

Difficulties in standardization arise due to:
- Mostly the active ingredient is unknown;
- Synergy between two or more compounds; [17].
- A general approach is to standardize extracts on the basis of the quantitative presence of one or more marker compounds. Still, presence of marker compounds alone may not assure consistency in the biological activities as non-reproducibility results from variations in the unknown active principle [18].

Even though pre-clinical screening *in vitro* assays are there but they are not ideal for routine quality control as they are:
- laborious,
- time consuming and

- expensive since high throughput assays are not always suitable.

Hence a rapid and comparatively economical technology needs to be developed.

1. Chromatographic technique such as Thin layer Chromatography (TLC), High Performance liquid Chromatography (HPLC) and High Performance Thin layer Chromatography (HPTLC) is commonly used for standardization. This can be said to give a chemical Fingerprint of the active markers present in the extract.

Features of TLC:
- Determination of small amounts of impurities
- Type of Adsorbent and method of activation
- For the Preparation and concentration of test and control samples
- Volume of the solution to be taken on the plate
- Mobile phase and the migration distance of the sample taken.
- Determination of the drying conditions and method of their detection
- Determination of R_f values, fluorescence and colour

HPTLC analysis

1 2 3 4

Lane 1, 2: Cinnamaldehyde
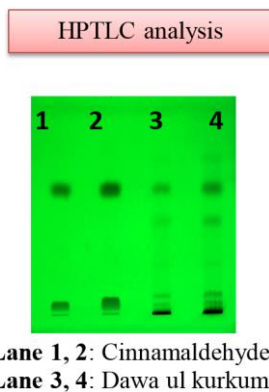Lane 3, 4: Dawa ul kurkum

Fig 3: HPTLC images at 245nm on Aluminium silica gel plates. Analysis of TLC is shown in Figure 3. HPTLC photos of Dawa-ul-kurkum ethanolic extract in n-Butanol: Acetic acid: water at 254 nm. L1-L2: Cinnamaldehyde, L3-L4: Dawa-ul-kurkum

TLC analysis

Cinnamomum zeylanicum

Fig 4: TLC plate. Thin layer chromatography fingerprint of *Cinnamomum zeylanicum* Aqueous Ethanolic extract.

Features of HPTLC:
- To Identify and detect adulterants in Unani product.
- To identify mycotoxins, pesticide content and in quality control of health food and herbs.
- Smaller quantity of mobile phase than in HPLC and a number of samples can be run all together.

- To continually detect chromatogram with the same or different conditions.
- Real-time assay of several components in a poly Unani formulation.
- To determine the phytoconstituents in crude drugs or Unani formulations such as bergenin, catechine and gallic acid in *Bergeniacilliata* and *Bergenialingulata.*
- No prior treatment of sample like filtration and degassing.
- Non-UV absorbing compounds detected by detected by post chromatographic derivatization.

Analysis of HPTLC is shown in Figure 4.

Comparison between HPTLC and TLC is shown in Table 1.

Table 1: Comparison between Thin layer Chromatography (TLC) and High Performance Thin layer Chromatography (HPTLC)

Factors	TLC	HPTLC
Type of chromatographic plates	Handmade/Pre coated	Pre coated
Adsorbent layer	200-250mm	100-150mm
Particle size	10-12μm	5-6μm
Application of sample	5-20μm	4-8μm
Shape of sample	Manual/ Semi automatic	Semi automatic
Spot shape	Spot	Band
Sample volume	1-10μl	0.1-2μl
No of sample/plate	15-20	40-45
Optimal development distance	10-16cm	6-8cm
Time of Development	Decided by mobile phase	40% less than TLC
Quantization	Manual	Manual/Instrumentation
Reproducibility of result	Difficult	Reproducible

Features of HPTLC:
- Quantification of Unani extracts
- Pharmaceutical analysis
- Food industry

Features of HPLC:
- To isolate and purify Unani compounds in pharmaceutical industry. There are two types of preparative HPLC:
- low pressure HPLC - typically under 5 bar and
- High pressure HPLC - pressure >20 bar.
- In HPLC one need to spend lesser time on the synthesis conditions.
- For high resolution, sensitivity and fast analysis.
- To determine tablet dissolution of pharmaceutical dosages.
- Determinations of shelf life of pharmaceutical products.
- Pharmaceutical quality control.
- Identification of forged drug products.

The use of **LC-MS** and **¹H NMR** Spectroscopic techniques for metabolite profiling is far better than the conventional methods as it gives a FP of many molecules simultaneously and there is no need to know which components are indicators of efficacy [19].

Then again the bio-electronic tongue that is able to biologically and chemically standardize without dependence on identification of a single active component or mechanism of action can be used to aid evaluation of quality control parameters of botanicals such as batch to batch variation etc [20].

To minimize sample preparation a non-destructive and quick technique such as VS can also be employed for standardization and quality analysis of Unani plants [21]. Integration of these state-of-the-art technologies could thus be applied to ensure modernization of Unani medicine.

References
1. WHO Report. WHO/EDM/TRM/2002. URL: http://www.wpro.who.int/health_technology/book_who_traditional_medicine_strategy_2002_2005. Geneva: World Health Organization, 2002, 19.
2. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of medical and Biological research. 2000; 33(2):179-89.
3. Capasso F. The medicinal plants in our time. Bollettino chimico farmaceutico. 1986; 125(9):322.
4. Taylor DA. Botanical supplements: weeding out the health risks. Environ Health Perspect. 2004; 112:A750-753.
5. Boullata JI, Nace AM. Safety issues with herbal medicine. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2000; 20(3):257-69.
6. Martins HM, Martins ML, Dias MI, Bernardo F. Evaluation of microbiological quality of medicinal plants used in natural infusions. International journal of food microbiology. 2001; 68(1-2):149-53.
7. Kneifel W, Czech E, Kopp B. Microbial contamination of medicinal plants-a review. Planta Medica. 2002; 68(01):5-15.
8. Reif K, Metzger W. Determination of aflatoxins in medicinal herbs and plant extracts. Journal of Chromatography A. 1995; 692(1-2):131-6.
9. Halt M. Moulds and mycotoxins in herb tea and medicinal plants. European Journal of Epidemiology. 1998; 14(3):269-74.
10. Efuntoye MO. Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. Mycopathologia. 1999; 147(1):43-8.
11. Elshafie AE, Al-Rashdi TA, Al-Bahry SN, Bakheit CS. Fungi and aflatoxins associated with spices in the Sultanate of Oman. Mycopathologia. 2002; 155(3):155-60.
12. Rai V, Agarwal M, Khatoon S, Rawat AK, Mehrotra S. Estimation of Co and Mn in some medicinal plants. Bulletin of environmental contamination and toxicology. 2001; 66(4):427-32.
13. Espinoza EO, Mann MJ, Bleasdell B, DeKorte S, Cox M. Toxic metals in selected traditional Chinese medicinals.Journal of Forensic Science. 1996; 41(3):453-6.
14. Pharmacopoeia Commission for Indian System of Medicine. Available from: http://www.indianmedicine.nic.in/writereaddata/mainlinkFile/File334.pdf. Visited on 30/10/2018.
15. Sharma SK. Infrastructure Status, Regulation & Quality Control of Indian Medicine. Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha, Homoeopath (AYUSH). New Delhi: Ministry of Health & Family Welfare, Government of India, 2006.
16. Briskin DP. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant physiology. 2000; 124(2):507-14.
17. Inui T, Wang Y, Deng S, Smith DC, Franzblau SG, Pauli GF. Counter-current chromatography based analysis of synergy in an anti-tuberculosis ethnobotanical. Journal of Chromatography A. 2007; 1151(1-2):211-5.
18. Whalley BJ, Wilkinson JD, Williamson EM, Constanti A. A novel component of cannabis extract potentiates excitatory synaptic transmission in rat olfactory cortex in vitro. Neuroscience letters. 2004; 365(1):58-63.
19. Holmes E, Tang H, Wang Y, Seger C. The assessment of plant metabolite profiles by NMR-based methodologies. Planta Medica. 2006; 72(09):771-85.
20. Ahmad M, Ismail Z, Chew OS, Islam AK, MdShakaff A. Development of multichannel artificial lipid-polymer membrane sensor for phytomedicine application. Sensors. 2006; 6(10):1333-44.
21. Schulz H, Baranska M, Belz HH, Rösch P, Strehle MA, Popp J. Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements. Vibrational Spectroscopy. 2004; 35(1-2):81-6.