QUALITY ANALYSIS OF TRADITIONAL UNANI FORMULATION DAWA- UL- KURKUM

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Abstract

Proper identification & standardization is mandatory to ensure the therapeutic efficacy of herbal drugs used for health ailments. Dawa-ul-Kurkum (DK) is an important Unani formulation that has been commonly used in the treatment of liver dysfunction, anorexia, ascites, and abdominal pain. This study is aimed to formulate standard manufacturing procedures for DK and to develop its organoleptic, physicochemical, and TLC standards. Dawa-ul-Kurkum is a semi-solid honey-based compound formulation that contains seven main ingredients. An in-house formulation was prepared and standardized in accordance with the National Formulary of Unani medicine (NFUM). The finished product, DK, was analysed for organoleptic, physicochemical properties, TLC& HPTLC. Standardization of DK was achieved by organoleptic study and physicochemical parameters such as loss on drying, extractive values, ash value, total reducing sugars, and heavy metal study. Physicochemical values could be used to establish and formulate procedures for standardization and quality control of Dawa-ul-Kurkum. Total fungal and bacterial counts were found to be within the permissible limit. Heavy metals like arsenic, mercury, lead, and cadmium was also found to be in permissible quantities. This study helps in determining the quality and purity of Dawa-ul-Kurkum and may serve as a standard reference for developing standard operating procedures for the quality control analysis of Dawa-ul-Kurkum.

Keywords: Standardization, Compound Formulation, Physico-chemical study, HPTLC, Quality control, and quality assurance

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1. Introduction

Due to the long history of clinical practice and fewer side effects, herbal medicines are gaining more and more popularity at the global level. The world is turning to the use of medicinal plants and products for the prevention of ailments. The need for alternative medicine has resulted in the growth of natural product markets and interest in Ayurveda, Siddha, and Unani (ASU) traditional systems of medicine. ASU systems offer different types of therapeutic approaches and natural products for the treatment of Liver disorders. A series of reviews undertaken by Bhatt et al provide major information about the contextual and clinical aspects of most potential plant drugs used singly or in combinations for the treatment of liver disorders[1-8]. Dawa-ul-Kurkum (DK) is an important Unani formulation mentioned in the National Formulary of Unani Medicines (NFUM), which has been commonly used in the treatment of liver dysfunction, anorexia, ascites, and abdominal pain[9,10]. The development of standard operating procedures and quality assurance/ control is an important factor and a basic requirement for drug development. Proper integration of ern scientific techniques and traditional knowledge is important. This study is an attempt to formulate standard manufacturing procedures for DK and to develop its organoleptic, physicochemical, and TLC standards.

2. Materials and Methods

Test drugs

Dawa-ul-Kurkum is a semi-solid compound formulation that contains seven main ingredients and honey as a base [Table 1].

Collection of samples

All the seven ingredients Zafran (Crocus sativa), Sumbuluttib (Nardostachys jatamansi DC), TajQalmi (Cinnamomum cassia), ShagofaIzkher (Cymbopogon jwarancusa), Mur (Commiphora myrrh), QustSheereen (saussurea lappa), and Dar Chini (Cinnamomum zeylanicum) were procured from minimum three reliable sources (authentic herbal drug suppliers from Mumbai, Pune and other parts of India). All the raw materials were separated from physical impurities and standardized as per the guidelines[11]. Honey was procured from the local market in Pune. The herbal material was authenticated in the Pharmacognosy Laboratory of BharatiVidyapeeth Deemed to be University and Savitribai Phule University, Pune. All the chemicals and solvents used were of analytical grade, and calibrated analytical instruments were used in the study.

Table 1: Ingredients, Botanical name, parts and quantity for preparation of Dawa-ul-Kurkum

S. No	Name of the Ingredient	Botanical name	Parts used	Quantity as per classics
1.	Zafran- Kumkum-Kesar	Crocus sativus L. [CS]	Stamens	1 part
2.	Sumbul-ut-Teeb–Jatamansi	Nardostachys jatamansi DC. [NJ]	Rhizomes	1 part
3.	Saleekha- Dalchini	Cinnamomum cassia BL. [CC]	Bark	1 part
4.	ShagufaIzkhar-Lamanjak	Cymbopogon jwarancusa (JONES) SCHULT. [CJ]	Whole plants	1 part
5.	Mur Makki-	Commiphora myrrha ENGL.[CM]	Resin	1 part
6.	Qust- Kushtha	Saussurea lappa C.B.CL. [SL]	Rhizomes	1 part
7.	Darchini- Dlchini	Cinnamomum zeylanicum BL.[CZ]	Bark	1 part
8.	Asal- Madhu	Honey	-	3 part
9.	Sharab-e-Musallas	Wine/Brandy	-	Q.S

Table 1: Presents the ingredients and the part of plant used in Dawa-ul-Kurkum formulation

Pharmaceutical Procedure

The formulation was prepared in two batches as per the National Formulary of Unani Medicine in the laboratory of Rajiv Gandhi Information Technology and Biotechnology, BVDU, Pune[12].Formulation has been coded as DK 1 and DK 2.

Authenticated raw materials were cleaned by removing foreign matter. The air-dried ingredients (except Mur makki and Zafran) were powdered separately and sieved through a mesh with a pore size of 150 (mesh no. 100, British Standard Sieve). Zafran was grounded in a china clay mortar with a pestle before mixing in the honey.1 gm. of Mur Makki powder was soaked in Sharab-e-Musallas (one part brandy mixed with three parts water) for 24 hours.After soaking in Sharab-e-Musallas for 24 hours, murmakki was powdered separately and gently in a metallic kharal (mortar). Mur makki and Sharab-e-musallas suspensions were combined with Asal (Honey).Powders of other ingredients are added to it progressively with continuous stirring.Whole mass was made homogenous by

stirringand the formulation was stored at room temperature.

Table 2: Details of Dawa- Ul- Kurkum preparation

Particulars	**Batch I- DK 1**	**Batch II- DK 2**
Quantity of each ingredient [gram]	15 gram	25gm
Total quantity of ingredients	105 gram	175 gram
Quantity of Brandy taken for soaking of Murmakki [ml]	5 ml Brandy+ 15 ml water	10 ml Brandy+ 30 ml water
Weight of Honey	320 gram	500gm
Total yield [gram]	450 gram	650gm
Total Duration	2 days	2 days
Date of starting	12/7/2020	30/03/2022
Date of completion	13/7/2020	31/03/2022

Figure 1 : Pharmaceutical steps of Dawa- Ul- Kurkum Preparation

Ingredients of Dawa- Ul Kurkuma

CZ- Cinnamomum zeylanicum CC- Cinnamomum cassia, NJ- Nardostachys jatamansi, SL- Saussurea lappa, CJ- Cymbopogon jwarancusa CS- Crocus sativus L, , , CM- Commiphoramyrrha ENGL

Distilled water

Sharab-e-Musallas

Asal- Honey

Mur Makki powder was soaked in Sharab-e-Musallas
(one part brandy mixed with three parts water) for 24 hours

Analytical Study

The samples of Dawa- Ul- Kurkum were subjected to organoleptic, microscopic, and physicochemical analysis.

Organoleptic study

The sample was analyzed for macroscopic characteristics such as appearance, colour, texture, odor, and taste[13].

Microscopic examination

The drug sample (5 g) was weighed and gently warmed in a beaker with 50 ml of water until completely dispersed in water. The supernatant was decanted after the mixture was centrifuged.The sediment was washed several times with distilled water before being centrifuged and the supernatant decanted. A few mg were placed in a watch glass and mixed with phloroglucinol and concentrated hydrochloric acid before being mounted in glycerin. The salient features of the drug were observed in different mounts[14].

Physico-chemical analysis

Physicochemical analysis involved the ash content, moisture content, water soluble extractive value, alcohol soluble extractive value, and reducing and non-reducing sugars of the formulation to develop preliminary analytical profiles[15,16,17].

Estimation of Heavy Metals

The procedure used for the analysis of heavy metals like lead, cadmium, mercury, and arsenic was as per WHO 1998 and AOAC 2005. Thermo Fisher Scientific M Series, 650902 V1.27 Model Atomic Absorption Spectrometer (AAS) was used for the analysis[18,19].

Analysis of Aflatoxin

The procedure was followed for the analysis of aflatoxins as per the Official Analytical Methods of the American Spice Trade Association (ASTA, 1997)[20].

Thin Layer Chromatography

Thin layer chromatography was carried out on a TLC plastic sheet of silica gel pre-coated with a layer thickness of 0.2 mm using Pet Ether (60–80) extraction. The chromatograms were scanned at 254 and 366 nm wavelengths, followed by spectral analysis. Plates were also scanned at visual range after spraying the visualisation reagent, 2% ethanolic sulphuric acid.

High performance thin layer chromatography fingerprinting

2 gm of each sample were carried out on silica gel 60 F254 percolated plates (0.2 mm thickness; from Merck India Limited, Mumbai). An applicator from Camag Linomat-5 was used for band application, and a photo documentation unit (CamagLinomat IV) was used for documentation of chromatographic fingerprints. The mobile phase used Toluene: Ethyl acetate (9:1). All the plates were developed over a distance of 8 cm in a saturated development chamber (20X10 cm with a stainless steel lid) and visualised under 254 nm.The tests were carried out at IDRL Pune and Agharkar Research institute, Pune

Observations

Organoleptic properties

The drug Dawa- Ul- Kurkum is dark brown in colour, semi-solid, has a characteristic odour, is pungent and sweet in taste, and is partially soluble in water.

Microscopical observations

The preparation reveals yellowish-brownish transparent pigments in the background under a high magnification microscope. Plant tissue material is in fragments, multicellular, brownish red material agglomerates, and oval-shaped transparent globules.

Physicochemical Standards

The moisture content, i.e., loss of weight on drying at 105 °C, total ash, acid insoluble ash, alcohol-soluble and water-soluble matter, the reducing sugar and non-reducing sugar were observed and tabulated (Table 2). The physicochemical analysis was showed its genuinity. Alfatoxin and heavy metal analysis revealed that the drug was free of these contaminants.

Table 3- Physico Chemical Parameters of Dawa- Ul- Kukum

Tests	Batch 1	Batch 2
Loss on Drying at 105 c. %W/W	22.29	26.11
PH	4.8	5.20
Total ash in %	0.92	0.71
Acid insoluble ash in %	0.39	0.15
Alcohol soluble extractive in %	59.45	27.06
Water soluble extractive in %	71.85	54.36
Reducing sugar	29.89	24.42
Non reducing sugar	6.57	5.5
Alfatoxin	Absent	Absent

Heavy metals		
Lead	2.79 PPM	2.06 PPM
Cadmium	0.287 PPM	0.085 PPM
Arsenic	Nil	Nil
Mercury	1.06 PPM	0.017 PPM
Copper	0.36 PPM	0.69 PPM

Thin Layer Chromatography

The formulation DK was next analysed for the presence of phytochemical constituents using TLC. Petroleum ether (60–80 C) extract was spotted on a silica gel "G" plate and developed with Toluene: Ethyl acetate (9: 1) as the mobile phase. All the samples showed identical spots in UV-254 nm, UV-366 nm and visible light (after being derivatized with the sulphuric acid reagent). In UV (254 nm), 366 nm, and visible light, it shows 7, 4, and 7 spots, respectively, with different Rf values. The TLC studies of petroleum ether extract are tabulated in table 3.

Table 4- Rf values of petroleum ether extract of Dawa- Ul- Kurkum

Solvent System	**Rf Values**					
	UV- 254 nm (07 Spots)		**UV – 366 nm (04 Spots)**		**with Ethanolicsulphuric acid reagent(07 Spots)**	
	Batch 1	**Batch 2**	**Batch 1**	**Batch 2**	**Batch 1**	**Batch 2**
Toluene: ethyl acetate (9:1 v/v)	**06 spots all blue** 0.12 0.19 0.30 0.40 0.50 0.65	**07 Spots all violate** 0.16 0.22 0.32 0.44 0.52 0.65 0.86	**04 spots** 0.39, [Blue] 0.47 0.61 0.87 [Flu. Blue]	**04 spots** 0.35, 0.69 [Blue] 0.51, 0.61 [Flu. Blue]	**06 Spots** 0.09 [Brown] 0.24 [Grey] 0.44 [Pink] 0.49 [Violet] 0.56 [Purple] 0.64 [Violet]	**07 Spots** 0.16 [Green] 0.44 [Yellow] 0.50 [Violet] 0.59 [Pink] 0.62[Blue] 0.67 [Grey] 0.79 [Brown]

High performance thin layer chromatography fingerprinting

High performance thin layer chromatography (HPTLC) was used to create fingerprint profiles of Dawa-ul-Kurkuma, as well as seven formulation ingredients and the markers kamepherol and Quercetin. The developed TLC plates were dried completely and detected under a UV cabinet system at 254 nm. Further, it was scanned with the densitometer CATS Planar Chromatography Manager system, which showed a typical densitogram, in which peaks appeared for the corresponding spots being detected in the densitometer. The peak areas of each component after separation and Rf values were recorded through the software. The corresponding densitograms, with peaks for the components shown in Table 4 figures 1 and 2, were obtained.

Table 5: HPTLC- Rf values of Quercetin, each ingredients &Dawa- Ul- Kurkuma
Abbreviations- NJ- Nardostachysjatamansi, SL- Saussurealappa, CZ- Cinnamomum zeylanicum, CC- Cinnamomum cassia, CJ- Cymbopogonjwarancusa, CS- Crocus sativus L. CM- Commiphoramyrrha , DK- Dawa- Ul- Kurkuma

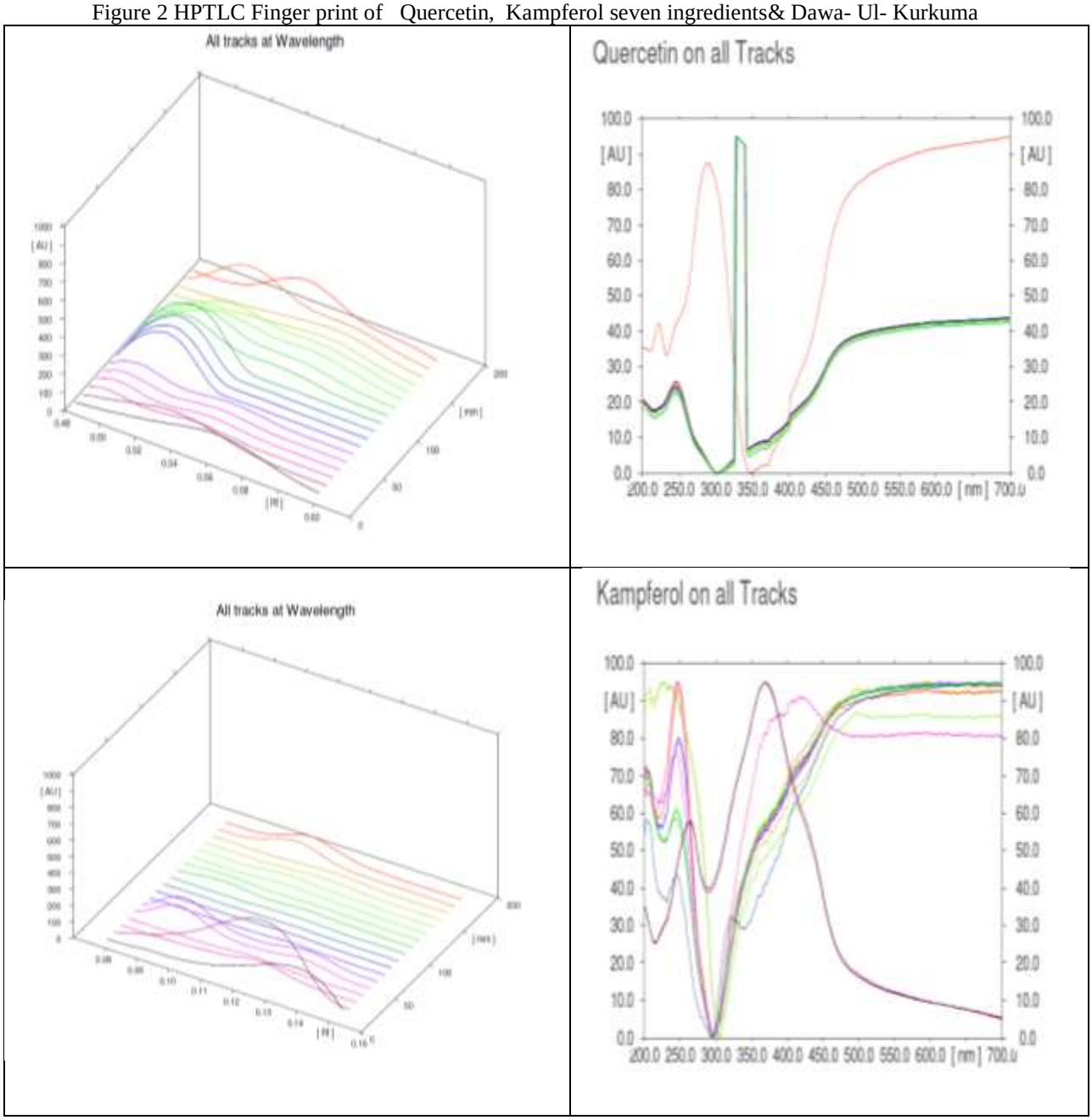
Track	**Samples**	**Rf value**	**Height**	**Area**	**Assigned substance**
1	Quercetin	0.62	2.6	5302.8	Quercetin
2	Quercetin	0.60	0.1	3797.3	Quercetin
3	NJ	0.58	0.1	576.7	Quercetin
4	NJ	0.58	0.1	603.5	Quercetin
5	SL	0.58	0.9	689.3	Quercetin
6	SL	0.59	0.0	801.1	Quercetin
7	CZ	0.54	1.7	5302.9	Unknown
8	CZ	0.54	0.3	5138.3	Unknown
9	CC	0.54	0.6	5547.3	Unknown
10	CC	0.55	1.5	7033.9	Unknown
11	CJ	0.60	0.4	1104.8	Quercetin
12	CJ	0.62	0.8	6347.5	Unknown
13	CS	0.62	1.4	2478.6	Quercetin

14	CS	0.62	2.2	2378.4	Quercetin
15	CM	0.61	0.2	3532.3	Quercetin
16	CM	0.62	0.2	3681.4	Quercetin
17	DK	0.62	3.5	8469.7	Unknown
18	DK	0.61	27.2	6694.0	Quercetin

Table 6: HPTLC- Rf values of Kampferol, each ingredients &Dawa- Ul- Kurkuma

Track	**Samples**	**Rf value**	**Height**	**Area**	**Assigned substance**
1	Kampferol	0.15	39.7	4085.6	Kampferol
2	Kampferol	0.15	0.6	8364.0	Kampferol
3	NJ	0.14	0.3	833.1	Kampferol
4	NJ	0.13	0.0	202.7	Kampferol
5	SL	0.15	0.2	575.0	Kampferol
6	SL	0.15	0.7	597.4	Kampferol
7	CZ	-	-	-	-
8	CZ	-	-	-	-
9	CC	-	-	-	-
10	CC	-	-	-	-
11	CJ	0.11 0.15	5.1 1.0	285.8 208.1	Unknown Kampferol
12	CJ	0.10	3.2	148.1	Kampferol
13	CS	0.14	0.4	326.0	Kampferol
14	CS	0.14	0.2	372.5	Kampferol
15	CM	0.13	0.5	296.8	Kampferol
16	CM	0.13	0.7	254.0	Kampferol
17	DK	0.13	0.8	979.2	Kampferol
18	DK	0.13	0.2	1027.0	Kampferol

Figure 2 HPTLC Finger print of Quercetin, Kampferol seven ingredients& Dawa- Ul- Kurkuma



3. Discussion

Various herbal products have been extensively used over the years in the management of liver diseases. Dawa-ul-Kurkum, a multifaceted Unani formulation, was analysed in the present work. An in-house formulation was prepared and standardized in accordance with the National Formulary of Unani medicine (NFUM). The finished product, DK, was tested for relevant physical and chemical parameters.

Pharmaceutical process

Mur makki is an oleo gum resin that is soluble in alcohol but not in water.Alcohols with better solubility in gum resin have larger solvent molecules and show higher alcohol–catalyst concentrations. The resin provides a greaer combined dipole moment and hydrogen bonding force interaction. Murmakki powder soaking in brandy for 24 hours could be blended or diluted without separating into two distinct substances.Fine powders of other ingredients and honey were added and stirred thoroughly to form a homogenous blend at room temperature to prevent possible volatilization.

Organoleptic analysis

The organoleptic properties such as smell, color, odor, taste, and consistency of food and other substances are experienced by the senses. Any changes give a primary indication of quality

variations and might be useful for distinguishing it from its substitutes and adulterants.

Physicochemical analysis

Established physicochemical standards give important information for further investigations and facilitate the identification of formulations. The moisture content of the drug reveals its stability and shelf life. The ash value determination forms the basis for judging the identity and cleanliness of a drug and provides information related to its adulteration by inorganic matter. The total ash content represents the amount of materials that remain after ignition, whereas acid insoluble ash represents the amount of silica that remains after ignition, specifically sand and siliceous matter.In the present study, DK was also evaluated for all these parameters.

Extractive values can be used to assess the consistency and amount of chemical constituents in a drug. The extractive values, such as water- and alcohol-solubility, indicate the amount of the active constituent and the bioavailability of the plant. A lower value indicates the presence of exhausted material. Formulation DK had a maximum aqueous-soluble extractive value compared to alcohol-soluble extractive values.

Reducing sugar, when chemically altered, can donate electrons to another molecule, which will change the colour and taste of food. The percentages of reducing sugar and non-reducing sugar are 24.42 and 5.5, respectively.

Aflatoxin and Heavy metal analysis

Aflatoxin, the secondary metabolite produced by the Aspergillus species, contaminates a variety of agricultural and food commodities. The formulation was tested for aflatoxins by the aflacord method, and none were found.

The heavy toxic metal contamination in plants could develop serious health problems as there is a narrow concentration range between the deficiency and toxicity levels of heavy metals in humans. The WHO has emphasised various standard techniques for the analysis of toxic heavy metals in plant products to ascertain their safety. Heavy metals were analysed by ICP-OES, and it was found that cadmium and mercury were below the detectable limit; and lead and arsenic were within the permissible limit, ensuring the safety of DK for human consumption.

TLC and HPTLC analysis

TLC and HPTLC studies are critical in both qualitative and quantitative analyses to ensure the purity of a compound or drug. The thin layer chromatography (TLC) study of selected plant samples was conducted using aluminium sheets of silica gel F254 (Merck®). All chromatograms were developed in a saturated chamber. The TLC studies of petroleum ether extract involved thesolvent system Toluene: Ethyl acetate (9: 1) resulted in the formation of different coloured spots. These RF values are indicative of the phytochemical constituents and provide important information about their polarity and the basis of their separation.

The HPTLC analysis of Dawa-Ul-Kurkum revealed the presence of Quercetin & Kampferol in varying concentrations, as shown in figures 2 and 3. Table 4 & 5 represents the overlay of the chromatograms of all tracks, at all measured wavelengths. The chromatogram scanned at 254 nm represents the presence of Quercetin in the samples NJ, SL, CJ,CS, CM in the range of 0.51 to 0.62 Rf values, while Kampferol present in NJ,SL,CJ,CS and CM in the range of 0.1 to 0.15 Rf values. When compare to Kampferol samples CZ and CC not showing any spectra.

The RF values calculated for the phytoconstituents present in the tested sample would be helpful in the identification of the unknown compounds by comparing them with the reference standards, and the concentration of the compounds could be determined.

Outcome of the Study

Quality Control and Quality Assurance of the Dawa-Ul-Kurkuma

This study established a set of quality parameters necessary for the standardization of the product. The assessment of quality parameters of herbal drugs is of profound importance in the determination of the efficacy and safety of the drugs.

Raw material Quality

The selection of appropriate-quality raw materials is crucial for the desired effect of herbal drugs. In the study, seven main ingredient samples were procured from at least three different sources. These were authenticated and their organoleptic, physicochemical, and TLC properties were investigated. The aqueous extracts of all plant drugs were evaluated for cytotoxic effects on the HepG2 cell line using the MTT assay. The plant drug samples that fulfil the standards of values for physicochemical parameters and Rf values with pharmacopoeia standards and have maximum efficacy for cytotoxic potential on the HepG2 cell line were taken for the preparation of the formulation.

Product standardization

Two different batches of Dawa-Ul-Kurkuma formulations were prepared by a qualified Unani medical practitioner as per the formula and instructions given in the NFUM. The establishment and validation of analytical parameters are

important requirements of quality assurance. The prepared formulations under study were subjected to physicochemical analysis, which, in conjunction with other parameters such as microscopic examination, heavy metal analysis, and aflatoxins contamination, is helpful in establishing the standard. It was seen that the total sugar content was above 35 percent in all the samples, which is sufficient for consistency and the preservation of the formulation, and it was also observed that about 2/3 of the sugar was reducing sugar and 1/3 was non-reducing sugar. The modern technique of HPTLC analysis was employed with respect to standardization and to separate the compounds that could be isolated for further studies.

No such standards are mentioned for DK. The above detailed step-by-step documentation in a scientific, logical, and sequential manner helps in developing a standard manufacturing procedure for DK. The study is likely to help with the quality assurance of DK and establish a set of quality parameters necessary for the standardisation of the product. It is also valuable in laying down standards for a multi-ingredient formulation. The quality standards will be used as diagnostic parameters for its identification and will aid in the maintenance of medicine quality.

4. Conclusion

The pharmaceutical process adopted in the current study can be considered a standard method. The preliminary physicochemical parameters and TLC study may be helpful as standards for further studies. The absence of microbial contamination and heavy metals reflects the quality and safety aspects of the formulation. The results obtained through this study were reliable, quick, and reproducible and could be used for routine quality control analysis of the formulations. It could help in maintaining the quality and batch-to-batch consistency of many important polyherbal formulations.This study may serve as a standard reference for developing standard operating procedures for the quality control analysis of Dawa-ul-Kurkum.

Conflict of Interests

The authors declare no conflict of interests.

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