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Pharmacognostical and Phytochemical Evaluation of a Unani Polyherbal Formulation: Dawa ul Kurkum by HPTLC

Meenakshi Gupta [1], Sajida Sumaiya [1,2], Sher Ali [3], Tanveer Naved [1], Archana Sharma [1], Ajaz Ahmad [4], Mohammed Sikander [5] and Maryam Sarwat [1,*]

1 Amity Institute of Pharmacy, Amity University, Noida 201301, Uttar Pradesh, India
2 Department of Ilmul Advia, School of Unani Medical Education and Research, Jamia Hamdard University, New Delhi 110062, Delhi, India
3 VC Office, Era University, Sarfaraz Ganj, Hardoi Road, Lucknow 226003, Uttar Pradesh, India
4 Department of Clinical Pharmacy, College of Pharmacy King Saud University, Riyadh 11451, Saudi Arabia
5 Department of Immunology and Microbiology, Biomedical Research, The University of Texas, Rio Grande Valley, 5300 North L Street, McAllen, TX 78504, USA
* Correspondence: msarwat@amity.edu

Abstract: Background: Dawa ul Kurkum (Duk) is a widely used Unani formulation. It consists of seven plant herbs, including stigmas of *Crocus sativus* L., rhizomes of *Nardostachys jatamansi* (D.Don) DC., the bark of *Cinnamomum cassia* (L.) J. Presl., shoot of *Cymbopogon jwarancusa* (Jones ex Roxb.) Schult., the resin of *Commiphora wightii* (Arn.) Bhandari, roots of *Saussurea lappa* (Decne.) Sch.Bip., and bark of *Cinnamomum zeylanicum* Blume. However, no study has been previously conducted to characterize this formulation. Thus, the present study was designed to carry out the pharmacognostic and phytochemical characterization of Duk. Methods: Duk was prepared following the protocols in *Bayaz e Kabeer* and *The National Formulary of Unani Medicine Part-I*. The characterization included organoleptic properties, fluorescence analysis, preliminary phytochemical screening, antioxidant activity, and active constituent profiling using HPTLC. Results: Evaluation of Duk showed the presence of carbohydrates, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarin, steroids, and phytosterols. The total phenolic and flavonoid content was 5.75 ± 0.23 mg GAE/g and 10 ± 0.18 mg QUE/g, respectively. HPTLC of Duk showed the presence of p-coumaric acid, cinnamaldehyde, citral, crocin, isovaleric acid, guggulsterone, and dehydrocostus lactone. Conclusions: Our findings supported the use of Duk as a conventional medicine, and these results could be used as a reference for the standardization of Duk.

Keywords: dawa ul kurkum; traditional knowledge; medicinal plants; phytochemistry; chromatography

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1. Introduction

In the last century, medical science has made remarkable advances worldwide. Overall mortality rates have reduced, life expectancy has improved, several new life-saving pharmaceuticals have been developed to aid in the battle against various infectious and other illnesses, and technological advancements have expanded the potential of contemporary science. Despite these incredible advancements and modernization in healthcare, quality treatment and its benefits have still not reached every door of the world [1]. The majority of the world's population, mostly in poor and underdeveloped nations, lacks access to modern medicine and relies on time-tested traditional/alternative or complementary systems of care [2,3].

Complementary and alternative healthcare and medical practices (CAM) are a varied set of medical and healthcare systems, procedures, and products that are currently not considered a part of modern medicine. Complementary medicine refers to therapies that supplement standard allopathic medicine and are used in conjunction with current treatments. Alternative medicine refers to therapeutic techniques utilized instead of standard

medicine to treat or improve illness outcomes. The fundamental principle of complementary and alternative medicine includes holistic treatment, which focuses on treating the individual [4]. The "WHO Traditional Medicine Strategy 2014–2023" clearly emphasizes the integration of traditional and complementary medicine to ensure qualitative, safer, effective, and universal healthcare [5]. In recent years, these approaches have gained enormous attention as they are safer, more reliable, and more cost-effective than synthetic forms of treatment [6,7]. Mainstreaming such medicinal plants is important as they offer fewer side effects and are rich in various secondary metabolites including alkaloids, flavonoids, glycosides, tannins, steroids, and saponins [7]. These metabolites are mainly responsible for their pleiotropic therapeutic effects, including anti-cancer activity [8].

Dawa ul Kurkum (Duk) is one such Unani polyherbal formulation, containing the stigma of *Crocus sativa* L., roots of *Nardostachys jatamansi* (Jones ex Roxb.) Schult. and *Saussurea lappa* (Decne.) Sch.Bip., the bark of *Cinnamomum cassia* (L.) J. Presl and *Cinnamomum zeylanicum* Blume, the aerial parts of *Cymbopogon jwarancusa* (Jones ex Roxb.) Schult., and the resin of *Commiphora wightii* (Arn.) Bhandari (Figure 1). It is prescribed by Unani practitioners for spleen disorders, liver dysfunction, anorexia, abdominal pain, ascites, and dropsy and is used as a renal, liver, and urinary bladder tonic and carminative [9,10]. The literature also reflects the hepatoprotective and immunomodulatory activity of Duk [11,12].

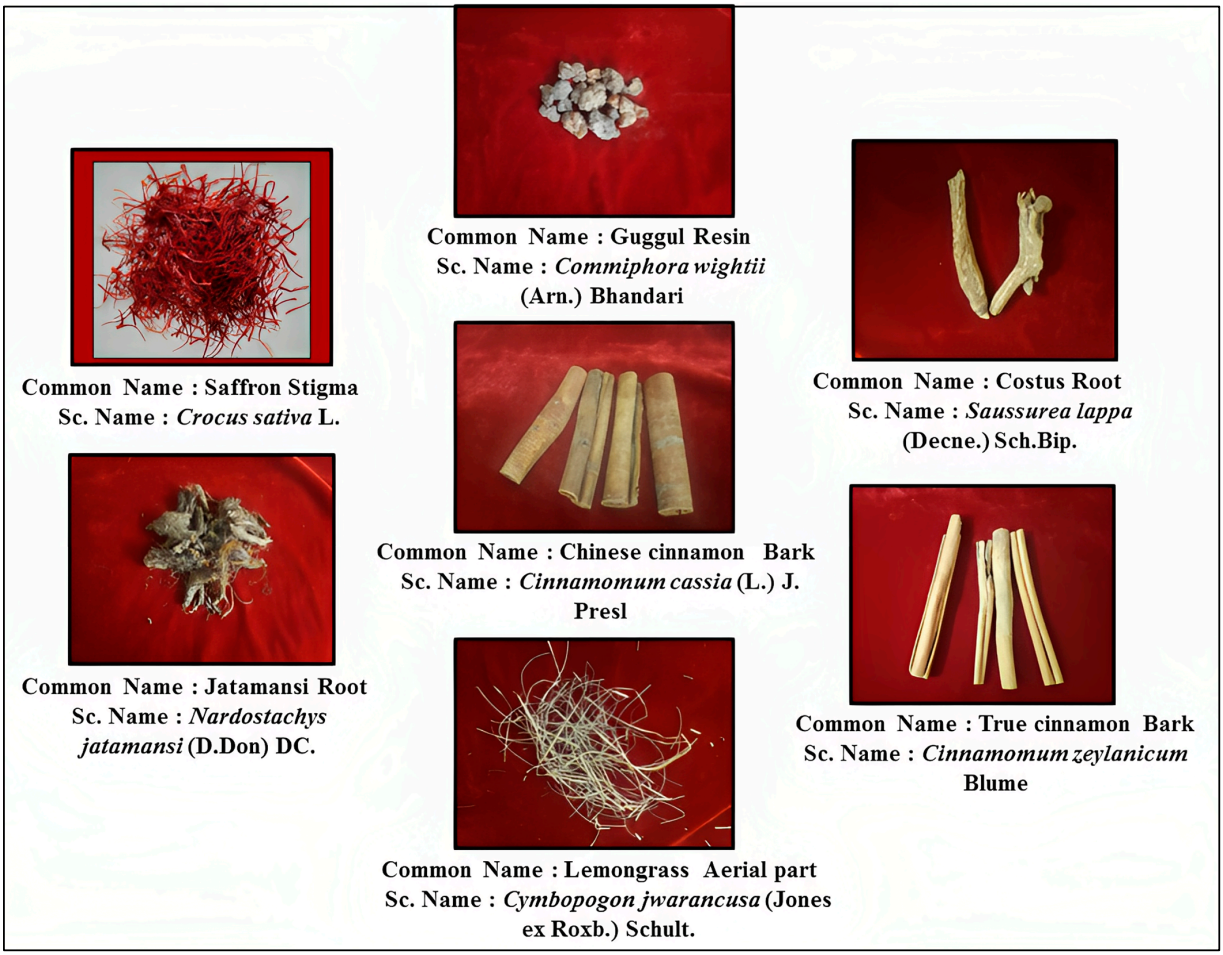

Figure 1. Representative pictures of the ingredients of our polyherbal formulation Duk.

The name "Duk" is due to its main ingredient Kurkum (Saffron gynacium). Saffron, a well-known spice, is widely used in human nutrition, cosmetics, perfumery, and as a dye. High-quality saffron contains approximately 30% crocins, 5 to 15% picrocrocin, and normally up to 2.5% volatile ingredients such as safranal. Along with these major bioactive

components, various other anthocyanins and flavonol glycosides have also been reported in various species of this genus [13]. Traditionally, saffron is used as an anti-inflammatory, antioxidant, anti-cancer, anti-depressant, anti-convulsant, hepatoprotective, neuroprotective, nephroprotective, cardioprotective, mutagenic and aphrodisiac agent [14–18]. Other constituents of Duk are also widely used due to their active constituents and pleiotropic properties. Jatamansi is used as a good stimulant, antispasmodic, tonic, laxative, and anti-epileptic and has been used in the treatment of a wide range of disorders, including the digestive system, circulatory system, nervous system, respiratory system, urinary system, reproductive system, and skin diseases [19]. These properties of jatamansi are mainly due to the presence of jatamansone, nardostachone, isovaleric acid, nardin, nardal, jatamnsic acid, spirojatamol, and various other active constituents [20]. Cinnamon is widely used in food preparations and industrial products such as candies, chewing gums, mouthwash and toothpaste. Cinnamon species exhibit various pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, wound healing, anti-HIV, anti-anxiety and antidepressant activities. It is also used to treat asthma, bronchitis, diarrhoea, headache, inflammation and cardiac disorders [21,22]. The essential oil of cinnamon is known to possess active constituents such as cinnamaldehyde, eugenol, caryophyllene, cinnamyl acetate and cinnamic acid [23]. In tribal areas, lemongrass is used as a flavouring agent for local cuisines. Moreover, the plant has also been used in traditional medicine as a blood purifier and in the treatment of rheumatism, cough, fever and cholera. Lemongrass contains active constituents such as citral, piperitone, δ-2-carene, α-phellandrene, citronellal, geranial and neral [24]. The major chemical constituents of guggul are z-guggulsterone, e-guggulsterone, guggul lignans, gugglu tetrols, mukulol, allylcembrol, z-guggulusterol, and e-guggulusterol, and it is used in digestive ailments, such as anorexia, flatulence, worm infestations, and piles [25]. Costus is known for its hepatoprotective, hypo-glycaemic, hypo-lipidemic, hypotensive and vaso-relaxing effect, anti-diabetic, anti-inflammatory, anti-microbial, antifeedant, anti-parasitic, anti-spasmodic, anti-ulcer and cholagogic, anti-cancer/tumour, immunomodulator, and bronchitic effects. The pharmacological activity of this plant is attributed to its active components: saussurine, costunolide, and dehydrocostus lactone [26].

Despite the varied use of Duk and its herbal components, no study has ever been conducted to explore this formulation on pharmacognostic grounds. Thus, the current investigation is aimed at assessing the physio- and phyto-chemical aspects of this formulation.

2. Materials and Methods

2.1. Collection of Plant Material, Drug Authentication, and Preparation of Duk

All the dried herbal drugs were purchased from Khari Bawli, Delhi, India (Table 1). The drugs were authenticated from NISCAIR, New Delhi, India and their voucher numbers provided were: *Cinnamomum cassia* (L.) J. Presl—NISCAIR/RHMD/Consult/2018/3156-05-6, *Cinnamomum zeylanicum* Blume—NISCAIR/RHMD/Consult/2018/3156-05-7, Cymbopogon jwarancusa (Jones ex Roxb.) Schult.—NISCAIR/RHMD/Consult/2018/3156-05-3, *Crocus sativa* L.—NISCAIR/RHMD/Consult/2018/3156-05-1, *Commiphora wightii* (Arn.) Bhandari—NISCAIR/RHMD/Consult/2018/3156-05-4, *Nardostachys jatamansi* (D.Don) DC.— NISCAIR/RHMD/Consult/2018/3156-05-2, and *Saussurea lappa* (Decne.) Sch.Bip.—NISCAIR/RHMD/Consult/2018/3156-05-5.

All the drugs (except *Crocus sativus* L., *Commiphora wightii* (Arn.) Bhandari and *Cymbopogon jwarancusa* (Jones ex Roxb.) Schult.) were crushed, ground, and sieved. *Crocus sativus* L. and *Commiphora wightii* (Arn.) Bhandari was crushed in a porcelain mortar pestle and *Cymbopogon jwarancusa* (Jones ex Roxb.) Schult. was ground using a mixer grinder.

Duk was prepared following the protocols of *Bayaz e Kabeer* and *The National Formulary of Unani Medicine Part-I* [9,10]. Briefly, a 70% ethanol extract was prepared from the powdered drugs by shaking and stirring at 50–60 rpm (RT, 24 h) and sonicating (30 min, <60 °C), and the extract was filtered (using Whatman no. 1). The total filtrate was concentrated

using a rotary evaporator (65 °C, 100–110 rpm) and lyophilized to dryness. The dried powder was weighed and kept in airtight glass vials (4 °C) until further use.

Table 1. Composition of Duk.

S. No.	Biological Name of the Drug	Common Name/ Unani Name	Part of the Plant	Weight (g)Used/ 50 g
1.	*Cinnamomum cassia* (L.) J. Presl	Chinese cinnamon/ Taj Qalmi	Bark	10
2.	*Cinnamomum zeylanicum* Blume	True cinnamon/Darchini	Bark	5
3.	*Cymbopogon jwarancusa* (Jones ex Roxb.) Schult.	Lemongrass/Izkhar	Aerial part	5
4.	*Crocus sativa* L.	Saffron/Zafran	Stigma	10
5.	*Commiphora wightii* (Arn.) Bhandari	Guggul/Murmakki	Resin	5
6.	*Nardostachys jatamansi* (D.Don) DC.	Jatamansi/ Sumbuluttib	Root	10
7.	*Saussurea lappa* (Decne.) Sch.Bip.	Costus/Qust	Root	5

2.2. Organoleptic Properties

The organoleptic property is the distinctive attributes or qualities of any drug perceived by sensory experiences. It refers to the evaluation of a drug based on its appearance, colour, odour, taste, and texture [27,28]. These properties were determined based on the methods mentioned by Wallis [29]. For the odour, a small amount of Duk was placed in a beaker and was slowly and repeatedly inhaled. The strength (none, weak, distinct, or strong) and sensation of odour (aromatic, fruity, rancid, musty, mouldy, etc.) were recorded. The taste was noted based on the classification as sweet, sour, pungent, bitter, astringent, or mucilaginous. The texture was recorded by gently placing the sample between the thumb and index finger and rubbing it.

2.3. Fluorescence Analysis

An amount of 1–2 mg Duk was placed on a microscope slide, and treated with different chemicals such as 1N NaOH (aqueous and alcoholic), 1N HCl, ammonia, 5% $FeCl_3$, 5% iodine, acetic acid, 1N HNO_3, and 1N H_2SO_4 and observed under daylight, short wave (254 nm) and long wave (366 nm) UV lights using a UV cabinet [30].

2.4. Preliminary Phytochemical Screening

The Duk was subjected to preliminary phytochemical screening to observe the presence or absence of phytoconstituents such as carbohydrates, tannins, saponins, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarin, steroids & phytosterols, phlobatannins, flavonoids and anthraquinones by the standard methods [Table 2]. The screening was done for the presence or absence of phytochemicals using diagnostic procedures such as colour concentration or precipitate formation. The findings were qualitative, with a positive '+' sign indicating the presence of phytochemicals and a negative '−' sign indicating their absence.

2.5. Quantitative Phytochemical Analysis

2.5.1. Total Phenol Content (TPC)

The Folin–Ciocalteu reagent (FCR; SRL Pvt. Ltd., Pune, India) method was used to determine the total phenolic content of Duk. 500 µL of Duk solution (1 mg/mL) was mixed with 3.5 mL of distilled water and 250 µL of FCR in a test tube and the solution mixture was left for incubation for 8 min at room temperature. An amount of 750 µL of 20% sodium carbonate solution was added to the test tube and incubated for 2 h. Finally, the absorbance was taken at 765 nm using a UV-visible spectrophotometer (LabIndia Instruments Pvt. Ltd., Mumbai, India) against the blank. The procedure was repeated three times and the total phenolic content was expressed as gallic acid equivalent [31].

Table 2. Identification test for the preliminary phytochemical screening of Duk.

S. No.	Test for	Procedure	Observation for the Presence
1.	Carbohydrates	2 mL of Duk solution + 1 mL of Molisch's reagent + Few drops of concentrated sulphuric acid	Purple or reddish colour
2.	Tannins	1 mL Duk solution + 2 mL of 5% ferric chloride	Dark blue or greenish colour
3.	Saponins	2 mL of Duk solution + 2 mL of distilled water; Shake for 15 min	1 cm layer of foam
4.	Flavonoids	2 mL of Duk solution + 1 mL of 2N sodium hydroxide	Yellow colour
5.	Alkaloids	2 mL of Duk solution + 2 mL of concentrated hydrochloric acid + Few drops of Mayer's reagent	Green colour or white precipitate
6.	Quinones	1 mL Duk solution + 1 mL of concentrated sulphuric acid	Red colour
7.	Glycosides	2 mL of Duk solution + 3 mL of chloroform + 10% ammonia solution	Pink colour
8.	Cardiac glycosides	0.5 mL of Duk solution + 2 mL of glacial acetic acid + Few drops of 5% ferric chloride + 1 mL of concentrated sulphuric acid	Brown ring at the interface
9.	Terpenoids	0.5 mL of Duk solution + 2 mL of chloroform + concentrated sulphuric acid	Red brown colour at the interface
10.	Phenols	1 mL Duk solution + 2 mL of distilled water + Few drops of 10% ferric chloride	Blue or green colour
11.	Coumarins	1 mL Duk solution + 1 mL of 10% NaOH	Yellow colour
12.	Steroids and phytosterols	1 mL Duk solution + 1 mL of chloroform + Few drops of concentrated sulphuric acid	Brown ring: Steroids Bluish brown ring: Phytosterols
13.	Phlobatannins	1 mL Duk solution + Few drops of 2% HCl	Red colour precipitate
14.	Anthraquinones	1 mL Duk solution + 10% ammonia solution	Pink colour precipitate

2.5.2. Total Flavonoid Content (TFC)

The aluminium chloride colorimetric method was used to determine the total flavonoid content of Duk. 500 µL of Duk solution (1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate aqueous solution, and 2.8 mL of distilled water. The yellow colour confirmed the presence of flavonoids. The test sample was incubated for 30 min at room temperature and absorbance was taken at 415 nm against the blank. The procedure was repeated three times and results were expressed as mg quercetin/g dry weight [31].

2.6. Evaluation of Antioxidants

2.6.1. DPPH Radical Scavenging Assay

An amount of 0.1 mM of methanolic solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH; Central drug House Pvt. Ltd., Delhi, India) was added to different concentrations (5–60 µg/mL) of Duk. The mixtures were vortexed and allowed to rest in the dark (RT) for 30 min. The decrease in the absorbance was measured at 517 nm through a UV spectrophotometer [32]. Ascorbic acid (SRL Pvt. Ltd., Pune, India) was used as a positive control. Percentage inhibition of DPPH radical was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(OD_{control} - OD_{sample})/OD_{control}\} \times 100$$

where $OD_{control}$ is the absorbance of the control and OD_{sample} is the absorbance of the DuK/Ascorbic acid. Percentage inhibition was plotted against concentration. All experiments were performed in triplicate.

2.6.2. Ferrous Reducing Antioxidant Capacity (FRAC) Assay

An amount of 1 mL of Duk solution at different concentrations (100 µg/mL–6.4 mg/mL), 2.5 mL of potassium buffer (0.2 M), and 2.5 mL of 1% potassium ferricyanide solution were added into the test tubes. The reaction mixtures were incubated for 20 min at 50 °C to

complete the reaction. After incubation, 2.5 mL of 10% trichloroacetic acid was added. Each mixture was centrifuged at 750 g for 10 min and 2.5 mL of supernatant was taken; to which 2.5 mL distilled water and 0.5 mL 0.1% ferric chloride solution was added. The solution mixture without Duk was treated as a blank. The absorbance was measured at 700 nm by a UV spectrophotometer against the blank. The experiment was repeated three times at each concentration [33].

2.7. Phytochemical Profiling of DuK through High-Performance Thin-Layer Chromatography (HPTLC)

A CAMAG TLC system composed of Camag Automatic TLC Sampler 4 (ATS4), TLC scanner 3, and win CATS 1.2.2 software (CAMAG CO., Muttenz, Switzerland) was used for HPTLC. Chromatography was performed on silica gel pre-coated aluminium plate 60 F254 plates (250 µm thickness; E. Merck, Darmstadt, Germany) using a mobile phase (mentioned in Table 3). The sample and standards (p-coumaric acid and guggulsterone: Natural Remedies Pvt. Ltd., Bangalore, India; dehydrocostus lactone, crocin, citral, and isovaleric acid: Sigma Aldrich Co. LLC., St. Louis, MO, USA; cinnamaldehyde: Tokyo Chemical Industry Co., Ltd., Chennai, Tamil Nadu, India) were spotted as bands of 6 mm width with a Camag 25 µL sampling syringe (Hamilton, Bonaduz, Switzerland) at an application rate of 50 nL/s. The plates were developed in twin trough development chambers. After development, the HPTLC plates were derivatized with anisaldehyde/vanillin sulfuric reagent and after drying; plates were heated in a hot air oven at 110 °C for 2 min.

Table 3. Optimization and validation for the presence of the principal active components in the polyherbal formulation Duk through HPTLC.

S. No.	Active Constituent	Solvent System	Ratio	Application Volume Standard	Application Volume Duk	Illumination	Rf Value	Post-Chromatographic Derivatization
1.	p-Coumaric acid	Toluene: Ethyl Acetate: Formic Acid	8:2:0.5	25 µL	15 µL	254 nm	0.28	Anisaldehyde Sulfuric Reagent
2.	Cinnamaldehyde	Toluene: Ethyl Acetate	9.5:0.5	15 µL	10 µL	254 nm	0.48	Vanillin Sulfuric Reagent
3.	Citral	Toluene: Ethyl Acetate	9.7:0.3	15 µL	15 µL	366 nm	0.62	Vanillin Sulfuric Reagent
4.	Crocin	n-Butanol: Water: Acetic acid	4:1:1	15 µL	10 µL	366 nm	0.28	Anisaldehyde Sulfuric Reagent
5.	Guggulsterone	Hexane: Ethyl Acetate: Acetic Acid	75:25:0.5	10 µL	10 µL	366 nm	z: 0.39 e: 0.29	Anisaldehyde Sulfuric Reagent
6.	Isovaleric acid	Toluene: Acetone	9:1	15 µL	15 µL	366 nm	0.11	Anisaldehyde Sulfuric Reagent
7.	Dehydrocostus lactone	Toluene: Ethyl Acetate	9:1	20 µL	20 µL	366 nm	0.56	Anisaldehyde Sulfuric Reagent

3. Results & Discussion

3.1. Organoleptic Evaluation

The Indian subcontinent possesses the richest and most diverse flora of blooming medicinal plants. Most of the world's population is primarily dependent on medicinal plants, with lifesaving pharmaceuticals being the most exceptional source. Approximately 80% of the world's population is interested in using traditional medicine based on plant components, and medicinal plants play an essential role in human medical treatment. They continue to be a substantial and valuable aid in working on individual well-being and the improved treatment of various ailments. Indigenous medicines play an essential role in primary health care and, therefore, their scientific evaluation is required for proper utilization [34]. Therefore, our polyherbal formulation was subjected to some standardization variables, such as organoleptic properties. The results were: Duk was powder in form, rust iron in colour, slightly rough in texture, and has a characteristic odour and taste.

3.2. Fluorescence Analysis

Fluorescence analysis is another standardization parameter for plant-based drugs. It determines the constituents of herbal medications and provides information about their chemical composition. Under suitable illumination conditions, the chemical constituents exhibit a fluorescence that is specific to them. In the case where chemical constituents are non-fluorescent, treatment with different chemical reagents can be done to attain the property [35]. Duk exhibited different colours on treatment with different chemical reagents. The colours were identified and noted in Table 4.

Table 4. Fluorescence analysis of the polyherbal formulation Duk.

S. No.	Treatment Conditions	Daylight	UV Light 254 nm	366 nm
1.	Duk as such	Reddish orange	Rust Brown	Blackish-brown
2.	Duk + 1N NaOH (aq.)	Brownish-yellow	Brown	Blackish-brown
3.	Duk + 1N NaOH (alc.)	Reddish-brown	Reddish-brown	Blackish-brown
4.	Duk + 1N HCl	Deep orange	Dark orange	Brown
5.	Duk + NH_3	Light brown	Light brown	Brown
6.	Duk + 5% Iodine	Deep brown	Brown	Brown
7.	Duk + 5% $FeCl_3$	Blackish-brown	Brownish-black	Black
8.	Duk + Acetic aid	Brownish-orange	Brownish-orange	Blackish- orange
9.	Duk + 1N H_2SO_4	Orange	Reddish-brown	Blackish-brown
10.	Duk + 1N HNO_3	Yellowish-orange	Reddish-orange	Reddish-black

3.3. Preliminary and Quantitative Phytochemical Screening and Antioxidant Activity of Duk

Since time immemorial, ethnomedicine has been widely used for the prevention and treatment of various diseases and disorders. The credit of this property can be attributed to the presence of various phytoconstituents and the antioxidant potential of these plants. Therefore, to support and expand the usage of Duk in traditional medicine systems, preliminary phytochemical screening and in vitro antioxidant activity were investigated [36]. It was observed that carbohydrates, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarin, steroids and phytosterols were present in Duk, whereas tannins, saponins, alkaloids, phlobatannins, and anthraquinones were absent in Duk (Table 5). As estimated by the FCR method and aluminium chloride colorimetric method, the total phenolic and flavonoid content was found to be 5.75 $\pm$ 0.23 mg GAE/g and 10 $\pm$ 0.18 mg QUE/g, respectively.

Table 5. Preliminary screening for the presence of secondary metabolites in DuK.

S. No.	Phytochemical Tests	Duk
1.	Carbohydrates	+ve
2.	Tannins	−ve
3.	Saponins	−ve
4.	Flavonoids	+ve
5.	Alkaloids	−ve
6.	Quinones	+ve
7.	Glycosides	+ve
8.	Cardiac glycosides	+ve
9.	Terpenoids	+ve
10.	Phenols	+ve
11.	Coumarins	+ve
12.	Steroids and phytosterols	Phytosterols
13.	Phlobatannins	−ve
14.	Anthraquinones	−ve

Significant evidence has accumulated to indicate that reactive oxygen species (ROS) and other oxidants play essential roles in developing various illnesses and diseases. The findings have shifted scientists' focus to an appreciation of antioxidants for disease prevention, therapy, and human health maintenance. The human body has an innate antioxidative system responsible for various biological activities, such as anti-mutagenic, anti-carcinogenic, and anti-ageing responses. Antioxidants stabilize or destroy free radicals frequently before they assault biological cell targets. Recently, there has been increasing interest in naturally occurring antioxidants because they are multifunctional and allow tremendous scope in rectifying ROS imbalance [37]. In the current study, the antioxidant activity of Duk has been assessed by DPPH and FRAP assay. DPPH solution measures the electron-donating ability of the herbal molecules. The activity is based upon the potential

of natural products to decolourize the DPPH solution as the change in colour is directly proportional to the concentration of antioxidant [38]. In the present study, it was observed that the DPPH radical scavenging activity was positively correlated to the concentration of the extract. At 5 µg/mL, the percentage of scavenging activity of Duk was 16.30% and it rose to 53.60% at a concentration of 40 µg/mL. At concentrations above 40 µg/mL, we observed a slight decrease in the activity ending with saturation. In the case of ascorbic acid (positive control), the percentage of scavenging activity at 5 µg/mL was 33.95% and 75.48% at a concentration of 60 µg/mL. The results of Duk and ascorbic acid were comparable at a concentration of 30 µg/mL (Figure 2a). The reducing power of Duk measured by FRAP was positively correlated to the concentration. The presence of reductants in Duk reduces the $Fe^{3+/}$ferricyanide complex to its ferrous form, thereby turning the yellow colour of the test solution to green [39]. Similar to the results obtained in the radical scavenging assay, the absorbance for the ferric reducing power of Duk increased from 0.10 to 3.75 at concentrations of 0.1 to 6.4 mg/mL (Figure 2b). The reducing power of Duk can be an indicator of its antioxidant potential.

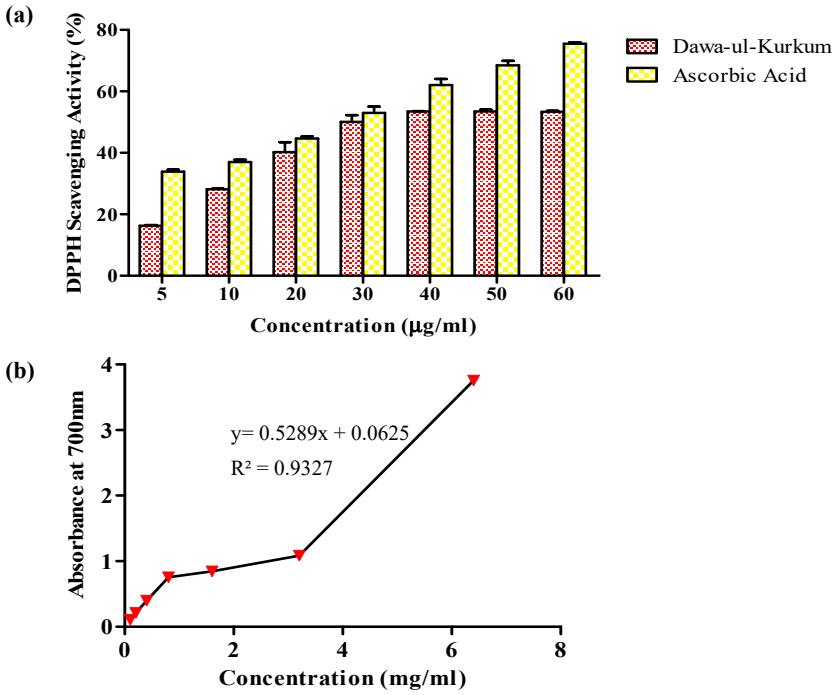

Figure 2. Antioxidant activity of Duk as evaluated with (**a**) DPPH, and (**b**) FRAP assays.

3.4. HPTLC of Duk Showed the Presence of All Seven Principal Active Metabolites of Its Constituent Herbs

The pharmacological activity of any herbal mixture is made possible by the presence of active constituents in it. Therefore, it is crucial to separate these metabolites, as they could be of clinical significance. HPTLC is one such simple, robust, reproducible, and popular technique for the identification of secondary metabolites in herbal extracts [40–42]. Through HPTLC, we have confirmed the presence of the main phytoconstituents of the seven herbal constituents in the polyherbal formulation prepared by us and this is the first report of the qualitative characterization of DuK. Present HPTLC fingerprinting data can be helpful in the authentication and identification of these active constituents of DuK in the performed solvent system.

The composition of the mobile phase for all the active constituents was optimized by testing a variety of solvent mixtures of different polarities and adding minute amounts of an acid or base. Well-defined spots were obtained when the TLC chamber was saturated with the respective mobile phase for 30 min at 25 ± 2 °C and the plates were post-derivatized with a suitable reagent (Table 3). In HPTLC, the heights corresponding to

the standards were seen at the desired Rf. A comparison of the characteristics of the height for standards and that of the sample (Figure 3) confirmed the presence of the main active constituents of the seven plants used to prepare Duk. These constituents are p-coumaric acid [*Cinnamomum cassia* (L.) J. Presl], cinnamaldehyde [*Cinnamomum zeylanicum* Blume], citral [*Cymbopogon jwarancusa* (Jones ex Roxb.) Schult.], crocin [*Crocus sativus* L.], isovaleric acid [*Nardostachys jatamansi* (D. Don) DC.], guggulsterone [*Commiphora wightii* (Arn.) Bhandari], and dehydrocostus lactone [*Saussurea lappa* (Decne.) Sch.Bip.].

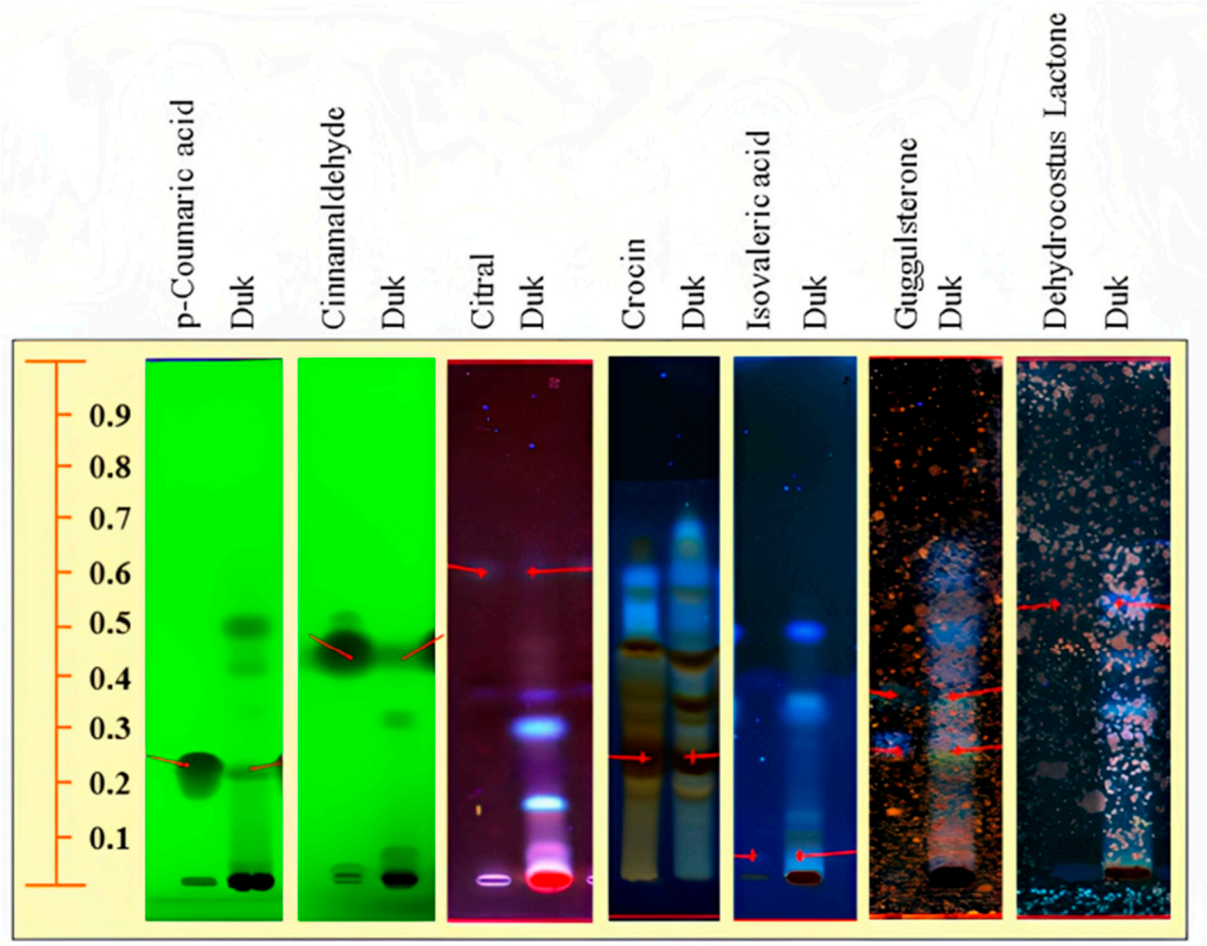

Figure 3. High-performance thin-layer chromatography and marker-based standardization of p-coumaric acid, cinnamaldehyde, citral, crocin, isovaleric acid, guggulsterone and dehydrocostus lactone in the developed polyherbal formulation. Red arrows shows the presence of the particular active constituent in the respective lane.

4. Conclusions

The current study analyzed the organoleptic and phytochemical characteristics, antioxidant activity, and HPTLC fingerprint of the polyherbal formulation. Duk showed the presence of various phytochemicals and possessed significant levels of phenols and flavonoids. In addition, Duk showed a critical anti-oxidant impact. The HPTLC fingerprint showed the presence of active constituents, such as p-coumaric acid, cinnamaldehyde, citral, crocin, isovaleric acid, e- and z-guggulsterone and dehydrocostus lactone. These findings will help in the standardization of this Unani preparation, Duk.

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