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DETECTION AND QUANTIFICATION OF STEROID ADULTERATION IN HERBAL MEDICINES USING TLC AND UV-SPECTROSCOPY

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ABSTRACT:

Herbal medicine has been a cornerstone of traditional healthcare systems for centuries, offering therapeutic benefits for a wide range of ailments. Despite their widespread use and perceived safety, herbal medicines face challenges related to contamination, adulteration, and regulatory oversight. This study focuses on the detection and quantification of steroid adulteration in herbal medicines using Thin Layer Chromatography (TLC) and UV-Spectroscopy techniques with eco-friendly solvents and reagents. The findings reveal that 5.96% (approximately 6.0%) of the analyzed herbal medicine samples contained adulterated steroids, with individual cases of adulteration remaining below 10%. The presence of steroids, such as prednisolone, in herbal formulations poses significant health risks, including metabolic, cardiovascular, dermatological,

and endocrine disorders. The study highlights the importance of stringent quality control measures and regulatory frameworks to ensure the safety and efficacy of herbal medicines. Consumers are advised to use herbal treatments prescribed by qualified practitioners and avoid unverified formulations that may lead to adverse health effects. This research underscores the need for increased awareness, improved regulatory policies, and advanced analytical techniques to safeguard public health from the risks associated with adulterated herbal medicines.

KEYWORDS: Herbal medicine**,** Steroid adulteration**,** Analysis, Quality control, Traditional medicine safety

INTRODUCTION:

Herbal medicine has been an integral part of human civilization for centuries, providing effective treatments for both acute and chronic ailments. With advancements in scientific research, ethnobotanical pharmacology has expanded, reinforcing the significance of plant-based remedies in modern healthcare. The accessibility, affordability, and perceived safety of herbal medicines contribute to their widespread use, particularly in developing nations where healthcare services are often limited (1-8).

In many cultures, traditional medical systems such as Ayurveda, Unani, Siddha, and Homeopathy have played a crucial role in shaping contemporary herbal medicine practices. The World Health Organization (WHO) estimates that approximately 75-80% of the population in underdeveloped regions relies on herbal treatments as their primary healthcare resource. The growing preference for natural remedies over synthetic pharmaceuticals is influenced by various factors, including cost-effectiveness, fewer side effects, and the holistic approach of herbal treatments (9-13).

The morphological and anatomical aspects of medicinal plants are essential in understanding their therapeutic properties. Herbal medicines, also referred to as phytomedicines or phytotherapy, consist of plant-derived substances that have not been chemically modified. They serve as a foundation for modern pharmacognosy, with numerous contemporary drugs originating from traditional herbal sources. Notable examples include aspirin (from willow bark), morphine (from the opium poppy), digoxin (from foxglove), and quinine (from cinchona bark) (14-19).

Despite the resurgence of interest in herbal medicine, certain challenges persist, including contamination, adulteration, and regulatory limitations. Contamination in herbal medicines may occur due to environmental pollutants, chemical additives, or microbial infections, compromising their purity and efficacy. Adulteration, whether intentional or accidental, further diminishes the quality of herbal products by introducing inferior or harmful substances. Regulatory measures are necessary to ensure the safety and standardization of herbal medicines, particularly in regions where they constitute a primary healthcare system (20-26).

In countries like Pakistan, the Unani system remains a dominant form of traditional medicine, with thousands of practitioners providing healthcare services in both rural and urban settings. Pakistan is also among the leading exporters of medicinal plants, indicating the economic significance of herbal medicine. However, the lack of comprehensive research, proper storage facilities, and formal training programs pose significant challenges to the development and regulation of the herbal medicine industry (26-32).

This manuscript aims to explore the historical, morphological, and pharmacological aspects of herbal medicine, highlighting its significance, challenges, and future prospects. By addressing

contamination, adulteration, and regulatory concerns, the study will contribute to the growing body of knowledge necessary for the advancement of herbal medicine as a viable and scientifically validated therapeutic option.

SAMPLE COLLECTION:

COLLECTION OF HERBAL MEDICINES SAMPLES:

Test specimens were collected from various markets in Lahore, as illustrated in Figure 1. The samples were sourced from different locations across the city, including Lohari Gate, Nishat Colony, Bhatta Chowk, Nishtar Colony, Awan Town, and Walton Road. A total of sixty quack formulations commonly used by patients were randomly selected from these areas. However, any drugs with known steroid content or allopathic ingredients were excluded from the study.

The final sample set consisted of forty-two quack formulations prescribed by local practitioners. These formulations were selected based on the following criteria: their constituents were unknown to contain steroids, they were self-prescribed, unlabeled, uncertified mixtures from unregistered Hakeems, or herbal products. The samples were used by individuals of any age or gender. The primary objective was to detect the presence of steroids adulterated in these herbal medicines.

The study was conducted as a cross-sectional analysis at the Department of Pharmaceuticals, Pakistan Council of Scientific and Industrial Research (PCSIR).

Figure 1: Different Samples of Herbal Medicine

PHYSICAL FORM OF HERBAL DRUGS:

Herbal medicines are collected in the form of powder, pills, tablets, majun, capsules, and liquid syrups. About 20 samples were in the form of powder, 10 were in the form of tablets, 2 samples were in the form of majun, 3 samples were in the form of capsules, 5 samples were in the form of pills and 2 samples were in the form of liquid syrups.

These medicines were consumed with water, milk, and engulf as it is.

CATEGORIES OF HERBAL MEDICINES:

These randomly obtained samples were categorized into seven groups. It was used to treat certain types of diseases.

Categories A: Samples were used to treat certain types of diseases i.e., Treatment of Kidney, Cough, Muscle Pain, Joint Pain, Aphrodisiacs, Asthma and Depression (Table 1).

These samples were collected from Abdullah Hakim & Pansar Centre, Walton Lahore.

Categories B: Samples were used to treat certain types of diseases i.e., Treatment of Diabetes, Arthritis, Kidney Stones, Cough, Muscle Pain, Asthma and Fever. These samples were collected from Ahmed Dawakhana, Bhatta Chownk Lahore (Table 2).

Table 1: List of Samples in Category A

List of Samples	Diseases to Cure
A-1	Treatment of Kidney
A-2	Treatment of Cough
A-3	Treatment of Muscle Pain
A-4	Treatment of Joint Pain
A-5	Treatment of Aphrodisiacs
A-6	Treatment of Asthma
A-7	Treatment of Depression

Table 2: List of samples in Category B

List of Samples	Diseases to Cure
B-1	Treatment of Diabetes
B-2	Treatment of Arthritis
B-3	Treatment of Kidney Stones
B-4	Treatment of Cough
B-5	Treatment of Muscle Pain
B-6	Treatment of Asthma
B-7	Treatment of Fever

Categories C: Samples were used to treat certain types of diseases i.e., Treatment of Cough, Fever, Asthma, Muscle Pain, Gout, Rheumatoid arthritis, and Aphrodisiacs. These samples were collected from Samiullah Matab & Dawakhana, Nishtar Colony, Lahore (Table 3).

Categories D: Samples were used to treat certain types of diseases i.e., Treatment of Cough, Systemic lupus erythematosus, Muscle pain, Asthma, Kidney, Systemic vasculitis and Gout. These samples were collected from Sadat Herbal Care Centre Awan Town, Lahore (Table 4).

Table 3: List of samples in Category C

List of Samples	Diseases to Cure
C-1	Treatment of Cough
C-2	Treatment of Fever
C-3	Treatment of Asthma
C-4	Treatment of Muscle Pain
C-5	Treatment of Gout
C-6	Treatment of Rheumatoid arthritis
C-7	Treatment of Aphrodisiacs

Table 4: List of samples in Category D

List of Samples	Diseases to cure
D-1	Treatment of cough
D-2	Treatment of Systemic lupus erythematosus
D-3	Treatment of Muscle pain
D-4	Treatment of Asthma
D-5	Treatment of Kidney
D-6	Treatment of Systemic vasculitis
D-7	Treatment of Gout

Categories E: Samples were used to treat certain types of diseases i.e., Treatment of Kidney stones, Inflammatory bowel disease, Muscle pain, Chronic obstructive pulmonary disease, Sciatica, Joint Pain and General weakness. These samples were collected from Baba Fareed Matab-e-Unani, Lohari Gate, Lahore (Table 5).

Categories F: Samples were used to treat certain types of diseases i.e., Treatment of Joint, Asthma, Kidney, Muscle, Cough, Kidney Stone and Aphrodisiacs. These samples were collected from Al-Saudia Matab & Dawakhana, Nishtar Colony, Lahore (Table 6).

Table 5: List of samples in Category E

List of Samples	Diseases to cure
E-1	Treatment of Kidney stones
E-2	Treatment of Inflammatory bowel disease
E-3	Treatment of Muscle Pain
E-4	Treatment of Chronic obstructive pulmonary disease
E-5	Treatment of Sciatica
E-6	Treatment of Joint Pain
E-7	Treatment of General Weakness

Table 6 List of samples in Category F

List of Samples	Diseases to cure
F-1	Treatment of Joint
F-2	Treatment of Asthma
F-3	Treatment of Kidney
F-4	Treatment of Muscle
F-5	Treatment of Cough
F-6	Treatment of Kidney Stone
F-7	Treatment of Aphrodisiacs

EXPERIMENTAL WORK:

EXTRACTION OF HERBAL MEDICINE:

Extraction of Herbal Medicine is performed by Maceration, Solvent Extraction Method and Decoction Methods.

SOLVENT EXTRACTION METHOD:

For the Extraction of Herbal Medicines, half of the samples followed the solvent extraction procedure. Take randomly obtained samples using a spatula and put the substance on butter paper. Now turned on the Analytical Balance (Mettler Toledo). Put the substance in the Internal

Calibration Automatic Electronic Analytical Balance (Max. 220g, Min. 10mg) and weigh the substance. Take 10gm of the obtained sample and put it in a separating funnel. The separating funnel (Pyrex 1L Globe-shaped) should be washed with the solvent i.e., Chloroform (Sigma-Aldrich). Add the amount of weighted substance in the funnel and add Chloroform in it. 50ml Chloroform was added to the sample and shook the funnel well. Wait for 10 minutes. Now took filter paper (Whatman filter paper 1) and filter the sample. Erlenmeyer flask (Pyrex 50ml) was used for this purpose. Filtrates contain extracted substances whereas residue was present on filter paper. Shook the flask well and wait for 5-10 minutes. Using a magnetic stirrer hot plate, heat the sample for some time under normal temperature. Cool the substance and wait for a couple of minutes. Put a wooden cork on the flask and keep the supernatant in the refrigerator. Transferred the following supernatant extracts in an amber glass tube vail. Follow the same protocol for the other half samples (figure 2).

Figure 2: Solvent Extraction Method

MACERATION METHOD:

Half of the samples followed the maceration method for the extraction process of Herbal Medicine. In this method, herbal medicine took using a spatula and weigh the substance by using Analytical balance/Platform balance. We took 10gm of the substance by properly weighing it. Add this sample to Beaker (Pyrex 50ml) and pour down Ethanol (Sigma-Aldrich Absolute Ethanol). 30 ml of ethanol was added to the sample, shook well and wait for a while. Covered the samples by using aluminum foil. Refrigerate the following samples for 3-5 days. Gently shake the substance a couple of times. After a few days, filter out the macerated substance with the aid of filter paper. Supernatant extracts were present infiltrate whereas residue settled on top of filter paper. Heated the following substance on a hot plate for a while. It was the purest form of supernatant extract. Ethanol was added and further heat. Now transfer the extract in a glass tube amber vail. Follow the same procedure for the other half samples.

DECOCTION METHOD

For the Extraction of Herbal Medicines, half of the samples followed the decoction method. Take randomly obtained samples using a spatula and put the substance on butter paper. Now turned on the Analytical Balance (Mettler Toledo). Put the substance in the Internal Calibration Automatic Electronic Analytical Balance (Max. 220g, Min. 10mg) and weigh the substance. Take 10gm of the obtained sample and put it in the beaker. Now add 3/4 volume of solvent

(ethanol/methanol/chloroform) about 30 ml in it. Heat the solution on the hot plate at 60-80^{o}C.Stirring the solution with stirrer for some time. Now took filter paper (Whatman filter paper 1) and filter the sample. Erlenmeyer flask (Pyrex 50ml) was used for this purpose. Filtrates contain extracted substances whereas residue was present on filter paper. Filtrate which is present in the beaker again heat till dry it into precipitates. Now add remaining 1/4 solvent of about 10 ml in it and again heat it until 5ml is remained. It was the purest form of supernatant extract. Follow the same procedure for the other half samples.

ANALYSIS OF STEROID BY USING THIN-LAYER CHROMATOGRAPHY:

For the analysis of steroids by using Thin-layer Chromatography, the following steps should be performed

FORMATION OF STANDARD SAMPLES OF STEROIDS:

STANDARD SAMPLE OF PREDNISOLONE:

In a dried test tube, a small amount of Prednisolone standard was taken. It is necessary to wash the test tube first. Its solubility was tested by adding a little amount of water to it. It was discovered that it was insoluble in water and that the solution was cloudy.

A small portion of Prednisolone standard was taken in a test tube. A small volume of eco-friendly ethanol was added, and its solubility was checked. It was found to be soluble in ethanol solvent and the solution was clear. After 24 hours its appearance must be checked again with the naked eye, the solution was found to be clear.

STANDARD SAMPLE OF DEXAMETHASONE

Dexamethasone standard was taken in a dried test tube. Add the small volume of methanol to it and note down its solubility. It was found to be soluble in methanol solvent and the solution was clear. After 24 hours its appearance must be checked again visually, the solution was found to be clear.

STANDARD SAMPLE OF CORTISONE

A dried test tube was used to collect the cortisone standard. Add the small volume of chloroform to it and note down its solubility. It was found to be soluble in chloroform solvent and the solution was clear. Put the standard solution on a hot plate and heat the substance. Standard Sample obtained in its purest form.

STANDARD SAMPLE OF BETAMETHASONE

Betamethasone standard was taken in a test tube. Add the small volume of chloroform to it. The standard sample should be heated on a hot plate for a while and observed its solubility. The sample solution was clear and obtained in its purest form.

STANDARD SAMPLE OF PREDNISONE

Prednisone standard was taken in a test tube and added small volume of chloroform in it. Solubility was checked, it may be noted that the sample is soluble and clear (figure 3).

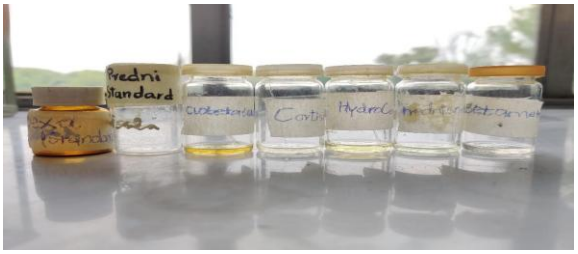


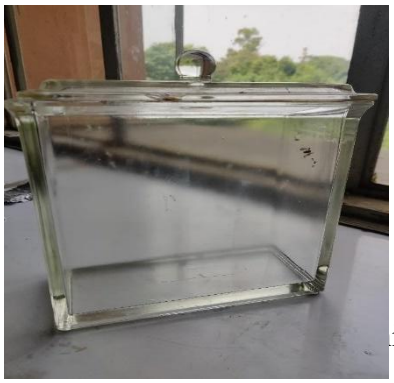
Figure 3: Standard Solution of Steroids

PREPARATION OF MOBILE-PHASE SOLVENT:

Mobile phase solvents used for the analysis of synthetic steroids by using TLC are

Methylene Chloride (Dichloromethane), b) Distilled Water, c) 1,4-dioxane

In the separating funnel, pour 50ml of methylene chloride solvent. In a separating funnel, combine 25mL distilled water and 25mL 1,4-Dioxane. Wait a moment after shaking the separating funnel vigorously. After some time, naked out noticed a distinct layer of organic and watery composition. Distinguish the aqueous and organic layers. In the Chromatographic tank, the organic layer was then filtered off (DESAGAHEIDELBERG). The mobile phase solvent was obtained in its purest form (figure 4a, b).



FORMATION OF THIN-LAYER CHROMATOGRAPHY PLATES:

The supernatant layer of the extracted sample was manually applied to the TLC plate using a capillary tube. Six to seven samples were analyzed simultaneously to save time and money. Standards for prednisolone, betamethasone, cortisone, prednisone, and dexamethasone were applied with the supernatant sample. The mobile phase used

was methylene chloride, distilled water, and 1,4-dioxane (50:25:25). A silica gel layer (20 cm x 20 cm) aluminum plate was used for the stationary phase. The chromatogram was developed by placing the plate in a glass TLC developing tank. The chromatogram was created up to 7-10 cm from the origin. The material was separated on these plates by elution in the mobile phase. After

drying, the plate is inspected with a UV lamp (254 nm) to see fluorescent spots on the steroid-positive sample, or 20% sulfuric acid in ethanol is sprayed onto the plate and then heated to 105 ° C for 10 minutes for coloring. A colored spot has been detected (figure 5a, b).

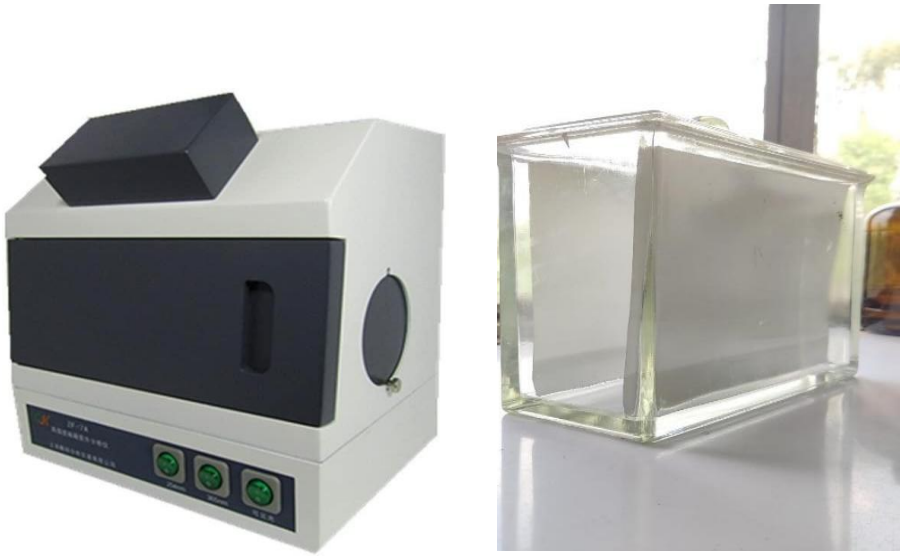


Figure 5: (a) UV-Lamp (b) Solvent Rise in Chromatographic Tank

FOR CATEGORY A TO F OF THE SAMPLES:

To make a TLC plate, we used a TLC plate and drew a line with the help of a pencil 1 cm apart from one end. Mark the spots of samples by using capillary tubes. Along with samples spotted the plate with standard samples of Prednisolone, Dexamethasone and Betamethasone. After 3 to 4 hours, 70-75% rise of mobile phase was seen. Dry the plate and saw under the UV lamp (figure 6-11a, b).

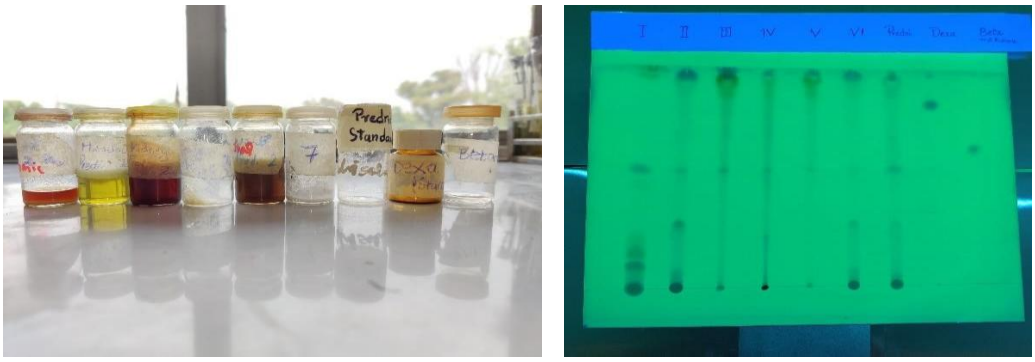


Figure 6: (a) Samples of Category A (b) TLC Plate for Category A

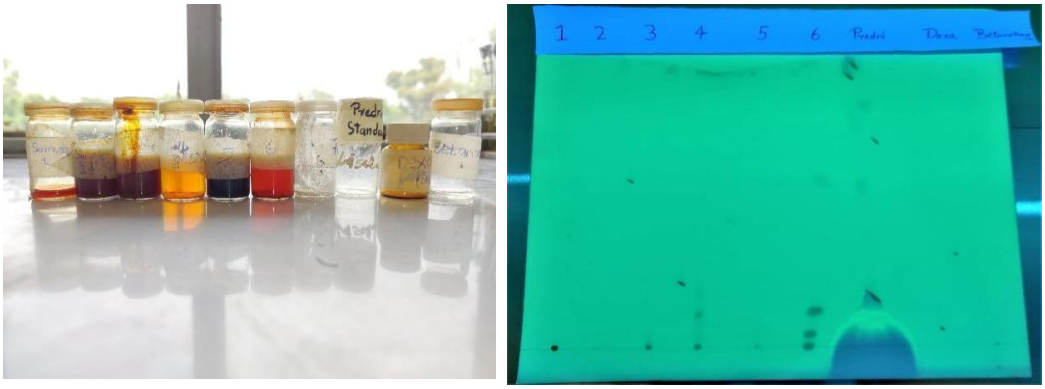


Figure 7 (a) Samples of Category B (b) TLC plate for Category B

Figure 8: (a) Samples of category C (b)TLC plate for Category C

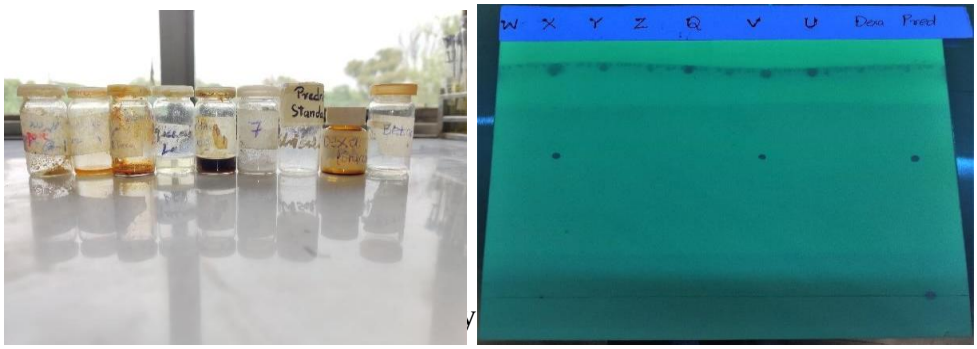


Figure 9: (a) Samples of Category D (b) TLC plate for Category D

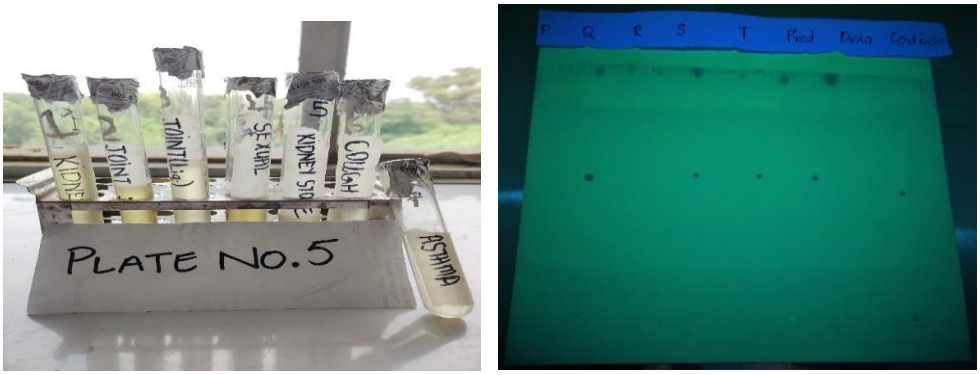


Figure 10: (a) Samples of Category E (b) TLC plate for Category E

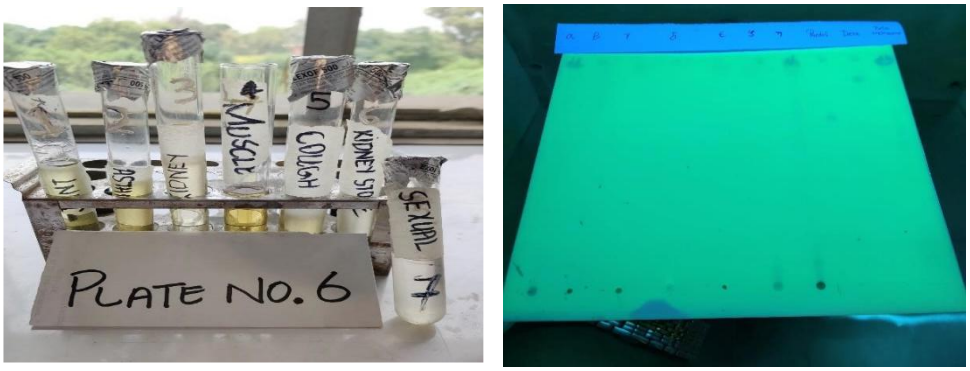


Figure 11: (a) Samples of Category F (b) TLC Plate for Category F

ANALYSIS OF STEROID BY USING ULTRAVIOLET-VISIBLE SPECTROSCOPY:

For the analysis of steroids using ultraviolet-visible spectroscopy, we had to make stock solutions of Prednisolone using a dilution formula. A calibration curve should be drawn to check the absorbance of Prednisolone.

PREPARATION OF STANDARD STOCK SOLUTIONS OF PREDNISOLONE:

Prednisolone Stock Solution (about 1000µg/ml):
Accurately weighed and transferred about 10mg of Prednisolone in a 10ml of dried volumetric flask. A small amount of ethanol was added and sonicated for about 2 minutes and then the volume was made up to 10ml with ethanol.
Prednisolone Standard Solution A (about 10µg/ml):

Add 1ml of stock solution in a volumetric flask and add 9ml of ethanol solvent in it. It formed a solution of about 10ppm (0.01mg/ml).

Prednisolone Standard Solution B (about 20µg/ml):

Add 2ml of stock solution in a volumetric flask and add 8ml of ethanol solvent in it. It formed a solution of about 20ppm (0.02mg/ml).

Prednisolone Standard Solution C (about 30µg/ml):

Add 3ml of stock solution in a volumetric flask and add 7ml of ethanol solvent in it. It formed a solution of about 30ppm (0.03mg/ml).

Prednisolone Standard Solution D (about 40µg/ml):

Add 4ml of stock solution in volumetric flask by mean of the pipette and add 6ml of ethanol solvent in it. It formed a solution of about 40ppm (0.04mg/ml).

Prednisolone Standard Solution E (about 50µg/ml):

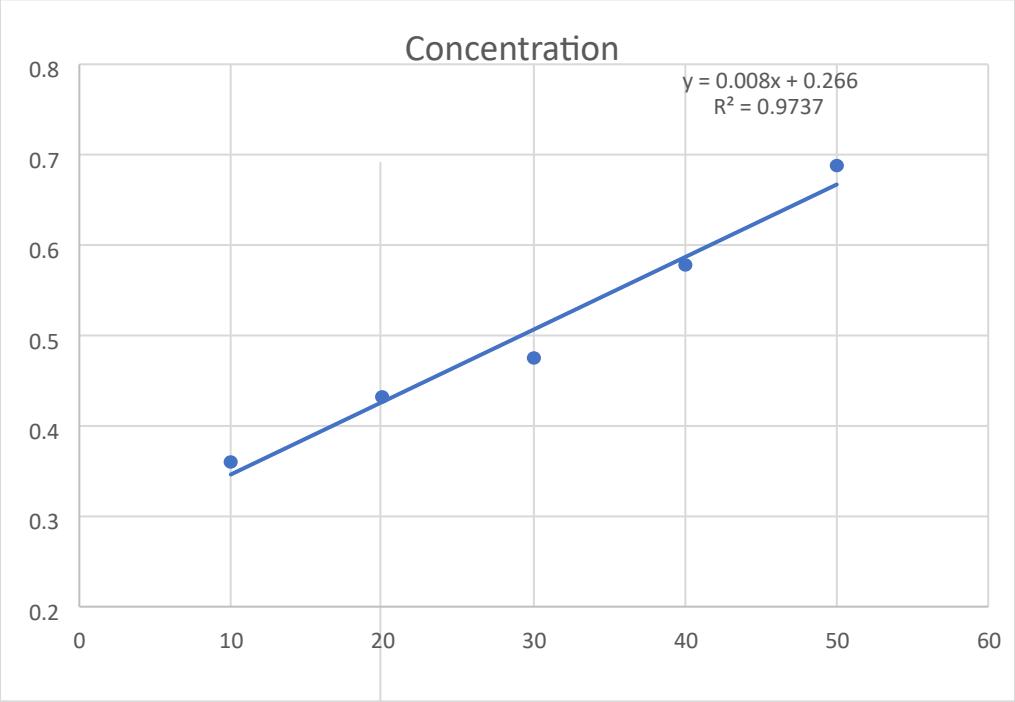
Add 5ml of stock solution in volumetric flask by mean of the pipette and add 5ml of ethanol solvent in it. It formed a solution of about 50ppm (0.05mg/ml).

With the help of a Single-Beam Spectrophotometer, determine the wavelength of stock solution and other standard solutions.

CALIBRATION CURVE:

A calibration curve was formed by plotting a graph between concentration (along the x-axis) and absorbance (along the y-axis). For a concentration range of about 10µg/ml to 20µg/ml**,** the developed method was found to be linear as the correlation coefficient was found to be 1.00**.** Lambda Max was found to be 243.1 nm. The R^2 value was 0.9737 (figure 12, Graph 1 & table 7).

Figure 12: Standard and Stock Solutions of Prednisolone



Graph 1: Calibration Curve of Prednisolone

Table 7: Relationship between Concentration and Absorbance

Number of Observation	Concentration	Absorbance
1	10	0.36
2	20	0.432
3	30	0.475
4	40	0.578
5	50	0.688

RESULTS:

EVALUATION OF STEROIDS BY THIN-LAYER CHROMATOGRAPHY:

Samples of herbal medicines in this study claimed to treat various diseases such as asthma, gout, diabetes, obesity, joint pain, muscle pain, rheumatism, and kidney diseases as well as to improve health. Forty-two to forty-five samples were collected from six different sources in the form of pills, tablets, liquid syrup, and majun. Steroids adulterated in samples were detected at quenching and violet spots on TLC plates using 254-nm UV light respectively. The R_f value of Prednisolone obtained from the mobile phase was 0.49 respectively. The LOD value of prednisolone was found to be 500µg/ml. Described procedure was applied for the determination of various samples of herbal medicines in Pakistan. Adulteration of steroids in samples was detected at a higher concentration than the LOD. The R_f value of prednisolone adulterated in samples was like those of steroid standards. Using a spike technique and comparing the TLC patterns of spots with those of the steroid standards were found to be adulterated with prednisolone (figure 13 & table 8).

Table 8: Contamination of Prednisolone in Herbal Medicines

Category	List of Samples	Name of Samples	Type of Samples	Suggested Use	Sample Source	Steroid Indication
A	**A-1**	Undeclared	Pills	Kidney	Abdullah Hakim & Pansar Centre, Walton Road, Lahore	Detected
	A-2	Undeclared	Syrup	Cough		Detected
	A-3	Undeclared	Powder	Muscle Pain		Detected
	A-4	Undeclared	Tablet	Joint Pain		Not Detected
	A-5	Undeclared	Powder	Aphrodisiac		Detected
	A-6	Undeclared	Capsule	Asthma		Detected
	A-7	Undeclared	Pills	Depression		----------
B	**B-1**	Undeclared	Powder	Diabetes	Ahmed	Not Detected

	B-2	Undeclared	Majun	Arthritis	Dawakhana, Bhatta Chownk, Lahore	Not Detected
	B-3	Undeclared	Capsule	Kidney		Detected
	B-4	Undeclared	Powder	Cough		Detected
	B-5	Undeclared	Powder	Muscle Pain		Not Detected
	B-6	Undeclared	Powder	Asthma		Detected
	B-7	Undeclared	Capsule	Fever		----------
C	**C-1**	Undeclared	Tablet	Cough	Samiullah Matab & Dawakhana, Nishtar Colony, Lahore	Not Detected
	C-2	Undeclared	Tablet	Fever		Not Detected
	C-3	Undeclared	Capsule	Asthma		Detected
	C-4	Undeclared	Capsule	Muscle Pain		Not Detected
	C-5	Undeclared	Powder	Gout		Detected
	C-6	Undeclared	Syrup	Rheumatoid		Not Detected
	C-7	Undeclared	Majun	Aphrodisiac		Not Detected
D	**D-1**	Undeclared	Pills	Cough	Sadat Herbal Care Centre Awan Town, Lahore	Not Detected
	D-2	Undeclared	Pills	S.L.E		Detected
	D-3	Undeclared	Pills	Muscle Pain		Not Detected
	D-4	Undeclared	Capsule	Asthma		Not Detected
	D-5	Undeclared	Powder	Kidney		Not Detected
	D-6	Undeclared	Capsule	Systematic vasculitis		Detected
	D-7	Undeclared	Powder	Gout		Not Detected
E	**E-1**	Undeclared	Capsule	Kidney Stones	Baba Fareed	Not Detected

	E-2	Undeclared	Capsule	Bowel Disease	Matab-e-Unani, Lohari Gate, Lahore	Detected
	E-3	Undeclared	Pills	Muscle Pain		Not Detected
	E-4	Undeclared	Majun	Pulmonary Disease		Detected
	E-5	Undeclared	Majun	Sciatica		Detected
	E-6	Undeclared	Tablet	Joint Pain		----------
	E-7	Undeclared	Pills	General		----------
F	**F-1**	Undeclared	Tablet	Joint Pain	Al-Saudia Matab & Dawakhana, Nishat Colony, Lahore	Not Detected
	F-2	Undeclared	Tablet	Asthma		
	F-3	Undeclared	Capsule	Kidney		
	F-4	Undeclared	Capsule	Muscle Pain		
	F-5	Undeclared	Pills	Cough		
	F-6	Undeclared	Pills	Kidney		Detected
	F-7	Undeclared	Syrup	Aphrodisiac		Detected

Table 8 showed the adulteration of prednisolone in a variety of herbal medicines. In most cases, samples unadulterated with steroids were found in the form of tablets, capsules, pills and majun. Even more dangerous was the detection of prednisolone in herbal pills, capsules, tablets, and powder which is used to cure arthritis, muscle pain, joint pain, asthma, and kidney. The most dangerous was the detection of prednisolone in almost all the herbal medicines belonging to any herbal center.

Samples that were adulterated or contaminated with steroid (Prednisolone), should be evaluated using UV-Spectroscopy. Standard samples were formed by using a dilution formula and making a stock solution of different concentrations. Lambda max value of samples was found to be 243.1 to 243.7 nm, respectively. This showed that the samples lambda max value corresponds to the standard prednisolone value.

To determine the percentage value of prednisolone, present in the sample solutions, it was divided with the standard solution value of prednisolone and multiplied by 100.

Formula: =absorbance of sample/absorbance of standard*purity of standard*100

Table 9: Percentage of Prednisolone in Samples (Category A)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone
A-1	0.75	10.0	7.5%
A-2	0.73	10.0	7.3%
A-3	0.57	10.0	5.7%
A-5	0.49	10.0	4.9%
A-6	0.68	10.0	6.8%

The Amount of Prednisolone present in samples was 0.75mg/ml, 0.73mg/ml, 0.57mg/ml, 0.49mg/ml, and 0.68mg/ml whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category A) was 7.5%, 7.3%, 5.7%, 4.9%, and 6.8% (table 9).

.**Table 10:** Percentage of Prednisolone in Samples (Category B)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone
B-3	0.93	10.0	9.3%
B-4	0.67	10.0	6.7%
B-6	0.75	10.0	7.5%

The amount of Prednisolone present in samples was 0.93mg/ml, 0.67mg/ml, and 0.75mg/ml, whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category B) was 9.3%, 6.7% and 7.5% (table 10).

Table 11: Percentage of Prednisolone in Samples (Category C)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone

C-3	0.66	10.0	6.6%
C-5	0.45	10.0	4.5%

The amount of Prednisolone present in samples was 0.66mg/ml and 0.45mg/ml, whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category C) was 6.6% and 4.5% (table 11).

Table 12: Percentage of Prednisolone in Samples (Category D)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone
D-2	0.26	10.0	2.6%
D-6	0.39	10.0	3.9%

The amount of Prednisolone present in samples was 0.26mg/ml and 0.39mg/ml, whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category D) was 2.6% and 3.9% (table 12).

Table 13: Percentage of Prednisolone in Samples (Category E)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone
E-2	0.265	10.0	2.65%
E-4	0.684	10.0	6.84%
E-5	0.736	10.0	7.36%

The amount of Prednisolone present in samples was 0.265mg/ml, 0.684mg/ml and 0.736mg/ml, whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category E) was 2.65%, 6.84% and 7.36% (table 13).

Table 14: Percentage of Prednisolone in Samples (Category F)

List of Samples	Amount of Prednisolone in Sample (mg/ml)	Amount of Standard Prednisolone (mg)	Percentage of Prednisolone
F-6	0.67	10.0	6.7%
F-7	0.88	10.0	8.8%

The amount of Prednisolone present in samples was 0.67mg/ml and 0.88mg/ml, whereas the amount of standard prednisolone is 10mg. The percentage of Prednisolone present in samples (Category F) was 6.7% and 8.8% (table 14).

DISCUSSION:

Prednisolone is a steroid medication used to treat certain types of allergies, inflammatory conditions, autoimmune disorders, and cancers. Some of these conditions include adrenocortical insufficiency, high blood calcium, rheumatoid arthritis, dermatitis, eye inflammation, asthma, and multiple sclerosis. It can be taken by mouth, injected into a vein or as eye drops.

The present study reports ecofriendly method use for the estimation of percentage of prednisolone in herbal medicines. Some people use herbal medicines for the cure of various diseases instead of medicated medicines because they thought that herbal medicines are safer than medicate medicines. But they never know that taking large number of herbal medicines are harmful for human health. Quick relief from the disease has various side effects on health.

Adverse reactions from the use of prednisolone include increased appetite, weight gain, nausea, increased risk of infection cardiovascular events, dermatological effects including reddening of face, bruising/skin discoloration, impaired wound healing, thinning of skin, skin rash, fluid buildup and abnormal hair growth, hyperglycemia, menstrual abnormalities and many more.

This study was conducted for finding the amount of prednisolone present in herbal medicines made by different quacks used for the treatment of various diseases like kidney problem, cough, arthritis and muscle pain etc. For this purpose, we gathered some samples of quack formulations for treatment of such kind of diseases from local pansar store because in medicines of local stores, hakeem use large number of steroids for quick relief from the disease. They don't think about its adverse effects on human health.

In this study, 42 samples were used for detection of prednisolone and divided these samples into 6 six categories randomly. These samples were in the form of pills, syrups, majun and tablet. Firstly, we carried out TLC method for detection of presence of prednisolone that in which samples prednisolone was present. Further for its contamination quantification is done by UV visible spectrophotometer.

Steroids adulteration in samples were detected at quenching and violet spots on TLC plates using 254-nm UV light respectively. The R_f value was obtained from the mobile phase was 0.49

respectively. Comparing spot length of different samples with standard prednisolone samples, it can easily find out in which prednisolone was present. For its quantification, samples were run along with positive samples of prednisolone in UV visible spectrophotometer and finding its absorbance. With the help of absorbance and concentration, R^2 was obtained which is0.9737. The percentage of prednisolone in category A was 7.5%, 7.3%, 5.7%, 4.9% and 6.8%. The percentage of prednisolone in category B was 9.3%,6.7% and 7.5%. The percentage of prednisolone in category C was 6.6 % and 4.5%. The percentage of prednisolone in category D was 2.6% and 3,9%. The percentage of prednisolone in category E was 2.65%, 6.84% and 7.36%. The percentage of prednisolone in category F was 6.7% and 8.8%.

The amount of prednisolone estimated in quack formulations was less than 10% in one tablet. This amount of steroid in one tablet is very large and it is harmful for human health. Taking prednisolone in excess amount can make more likely to get infections. If anyone take prednisolone for more than 3 weeks on high dose, don't stop prednisolone suddenly, it is very much harmful for him, it causes various side effects like nausea, heart burn, sleeping disturbances, menstrual cycle disturbance etc. Its use can be reduced slowly. Due to its adverse effect on human health, people should avoid ingesting prednisolone in excess amount by using quack formulations.

The Quack Formulation was an alternative treatment to the patients to cure their diseases. Many people were using quack formulation in this modern era. More than 60% of Asians use herbal medicines for health or the treatment of various diseases. According to WHO reports, across the world, 10-30 % of pharmaceuticals were counterfeit or adulterated, 15-20% of the medicines sold in India were adulterated and in China, 14.1% of the samples tested were either adulterated or substandard.

Excessive doses and long-term usage of medications that are contaminated with steroids may lead to many adverse effects. Although steroids are prescribed by allopathic physicians and herbal physicians for joint pains, dermatitis, and chronic obstructive airway diseases but their doses are well monitored to avoid any side effects. The trends of the utilization of QF in the form of powders, tablets and syrups were higher than creams. The contamination of steroids was found higher in tablets and powders than in other quack formulations. Herbal medicines sold by quack physicians were contaminated with steroids. The steroid mainly comprising of prednisolone was adulterated in the tablets and powders mostly prescribed by Hakeem. So, quackery formulations may be avoided or screened for steroids before use to avoid toxicity. People should be advised to use medicated medicines instead of quack formulations.

CONCLUSION:

A simple, reliable, and effective TLC method, along with UV-Spectroscopy techniques using eco-friendly solvents and reagents, was developed for the qualitative and quantitative analysis of steroids adulterated in various herbal medicine dosage forms. The study successfully identified the presence of steroids such as prednisolone in adulterated herbal medicines using the developed TLC method, while contamination levels were quantified through UV-Spectroscopy. These methods provide an economical approach for routine quality control of herbal medicines.

The results revealed that 5.96% (approximately 6.0%) of the total forty-two tested samples were adulterated with steroids. Although the individual percentage of steroid-adulterated samples remained below 10%, this level of adulteration poses a significant risk to human health. It is crucial for consumers to recognize the dangers of steroid-adulterated herbal medicines and to use only

medications prescribed by registered Hakeems or physicians. Excessive consumption of steroids can lead to severe health complications, including increased appetite, weight gain, nausea, heightened infection risk, cardiovascular issues, skin disorders, impaired wound healing, hyperglycemia, and menstrual irregularities.

To safeguard public health, individuals should avoid herbal formulations from unverified sources that promise quick relief but may cause long-term harm. Raising awareness about the risks of steroid-adulterated herbal medicines and promoting stringent regulatory measures can help mitigate these health risks. Ensuring proper quality control and adherence to safety standards in herbal medicine manufacturing is essential for maintaining their therapeutic benefits without compromising consumer health.

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