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STANDARDIZATION OF A HERBAL MEDICINE- *SWERTIA CHIRAYITA* LINN.

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ABSTRACT

There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. Medicinal plants and their products provide an effective source of treatment for various health ailments. For their appropriate utilization correct identification, authentication and quality control are essential, so as to ensure safety, efficacy and reproducibility in their therapeutic effect. Therefore, for the present study *Swertia chirayita* a well known herbal drug has been selected for its physicochemical and phytochemical standardization using Pharmacopoeial Guidelines. The parameters studied are ash values: total ash 2.40 ± 0.00 (0.48)%, acid insoluble ash 0.49 ± 0.00 (0.09)%, water soluble ash 3.12 ± 0.00 (0.62)%, sulphated ash 0.86 ± 0.01 (0.17)%; moisture content 8.37 ± 0.01 (0.83)%, loss on drying 8.69 ± 0.01 (0.86)%; pH value at 1% solution is 5.49 ± 0.00 and at 10% aqueous solution is 5.03 ± 0.01 ; melting range $80-90^{\circ}\text{C}$; solubility: water soluble extractive 5.89 ± 0.32 (1.19)%, and alcohol soluble extractive 3.86 ± 0.40 (0.75)%; bulk Density 0.67 ± 0.01 ; Crude Fiber Content 4.80 ± 0.07 (0.48)%, alkaloid content 11.53 ± 0.15 (1.15)%. On phytochemical analysis it revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols and proteins. Besides this determination of extractive values in different organic solvent, thin layer chromatography, fluorescence analysis of successive extract of powder drug, IR Spectral studies had also been done. As many parameters in this study provide one of the earliest data in standardization of Chirayita specifically: IR Spectral study, Fluorescence analysis, Crude fibre content, alkaloid assay. This study will help in setting down Pharmacopoeial standards for future reference in determining the quality and purity of *Swertia chirayita* Linn.

Keywords: *Swertia chirayita* Linn., Standardization, TLC.

INTRODUCTION

The Unani system of medicine (USM) is entirely based on the drugs of natural source and majority of the drugs are of herbal origin. And like any other system of medicine the efficacy of USM also depends on the potential and purity of drugs used. With the tremendous increase in the global use of medicinal plants, several concerns regarding the efficacy and safety of the herbal medicines have also been raised. Hence it has become priority to standardize the drugs to have uniform efficacy and safety measures so as to ensure regular supply of medicinal plant materials with good quality. So, it

is necessary to get assure of the identity, purity and quality of the drugs used. The existence of several common names for the same plant species in different areas may confuse end users for selection and utilization of a genuine drug. The other plants substituted for Chirayita are several species of *Swertia* are used as substitutes and adulterants of *S. chirayita*. The most important being *S. angustifolia*, *S. corymbosa*, *S. decussata*, *S. densifolia*, *S. paniculata*, *S. trichomata*; *Andrographis paniculata*, roots of *Rubia cordifolia*¹⁻⁶ So, the present study was done to provide a standardized

physico-chemical and phyto-chemical profile for *Swertia chirayita* on the basis of pharmacopeial guidelines for standardizing herbal drugs.

***Swertia chirayita* Linn. (Family: Gentianaceae)**

S. chirayita Linn. was first described by Roxburgh under the name of *Gentiana chirayita* in 1814. Ainslie notices it, and remarks that it appears to be much used in Bengal; it was probably rather a scarce drug in southern India in his time, as he says about it. In England it began to attract attention about the year 1829⁶ and in 1839 was introduced into the Edinburgh Pharmacopoeia. It is now official in the British and Indian Pharmacopoeia, and is generally accepted as a valuable bitter tonic. It is a native of temperate Himalayas, found at an altitude of 1200–3000 m (4000 to 10,000 ft), from Kashmir to Bhutan, and in the Khasi hills at 1200–1500 m (4000 to 5000 ft). It can be grown in sub-temperate regions between 1500 and 2100 m altitudes.^{1,7} It is used as anti-diarrhoeal, anti-helminthic, anti-inflammatory, anti-leucorrhic, antipyretic, anti-rheumatic, in anaemia, in burning sensation, cures leucorrhoea, diuretic, galactogoue, cholagogue, laxative, mild febrifuge, stomachic, thirst quenching, used in all types of fevers especially chronic and intermittent fevers, scabies and other skin diseases in traditional medical system. It enjoys a special reputation in western India as a remedy for bronchial asthma and in liver disorders.⁸⁻¹³ It is reported to possess a significant antibacterial efficacy against resistant strain MRSA (Methicillin Rensitive Staphylococcus aureus) by Latif *et al.*, 2011¹⁴ and various gram negative strains by Sumbul *et al.*, 2012.¹⁵

Although, the large continuous pith, opposite leaves and bicarpellary, unilocular fruits, dark colour and intense bitter taste are sufficient to distinguish *S. chirayita* from other species of the same genus and from plants belonging to families not possessing these characters⁶ but it is considered as a necessary step to carry on its Standardization as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially to determine the purity and quality of

the drugs official to it as in National Pharmacopoeia.

MATERIAL AND METHODS

Collection of Plant Material

Whole herb of *S. chirayita* was procured from local market of Aligarh city and was properly identified from the literature available and the Pharmacognosy Section, Department of ILMU Advia, Aligarh. The sample is preserved in the Herbarium of the Dept. for future reference (V.No.SC-0100/09-G). It was dried at optimum temperature and further crushed and sieved to coarse powder mechanically and stored in air tight container for study (Figure-1).

Organoleptic Parameters

The colour, taste, odour were noted which provide first hand information.

Physico-Chemical Analysis

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis include the determination of ash value, melting point, moisture content, pH value at 1% and 10% solution, solubility, bulk density, loss on drying. These were carried out as per guidelines of WHO and Govt. of India.¹⁶⁻¹⁹

Phytochemical Analysis

Phytochemical studies of the plant preparations are necessary for standardization, as it helps in understanding the significance of phyto-constituents in terms of their observed activities. Phytochemistry also helps in standardizing the herbal preparations so as to get the optimal concentrations of known active constituents, and in preserving their activities. The analysis include the determination of the extractive values in different organic solvents, qualitative analysis of the chemical constituents present in the drug sample.²⁰⁻²¹ Fluorescence Analysis of the powdered drugs and successive extracts (FTAR Analysis), crude fibre content, alkaloid estimation.²²⁻²⁴

IR Spectroscopic Study

For this alcoholic extract of the drug was obtained by refluxing powdered drug (5.0 g) with absolute alcohol (50 ml) for 5 hrs and removing the solvent under reduced pressure. The IR spectrum of alcoholic extract was determined in KBr pellets with Perkin Elmer 1600 FTIR spectrometer.²⁴

Thin Layer Chromatography

TLC analysis was conducted using different organic solvent systems in percolated silica gel 60F254 TLC plates. Thin Layer Chromatography of the extract of the test drug was carried out by. Spotted TLC plates were exposed to Iodine vapours in Iodine chamber and then heated at 105⁰ C in oven for 10 minutes; plates were visualized in day light and UV short and long wavelength. The R_f value of spots was determined by the given formulae.¹⁶⁻¹⁹

$$R_f \text{ value} = \frac{\text{Distance travelled by the Spot}}{\text{Distance travelled by the Solvent}}$$

OBSERVATIONS AND RESULTS

Organoleptic Characters

The powder of the dried herb of *S. chirayita* was dark green with characteristic bitter odour and taste, summarized in table-1.

Physico-Chemical Constants

Different Physico-chemical constants were determined three times and then average values depicted in table-2.

Phyto-Chemical Analysis

The phyto-chemicals present in the drug were qualitatively analysed by different chemical tests and results are given in table-3.

Qualitative Analysis of the Phyto-Chemicals

Qualitative Analysis of the phyto-chemical reveals the presence of alkaloids, carbohydrates, proteins, amino acids, phenols, sterols, glycosides, flavonoids, tannins, resins, sterols/ terpenes and volatile oil presented in table-4.

Florescence Analysis

Florescence analysis under UV light is sometime very characteristic for a drug. As many drugs and the constituents present in the drug emit specific colour when they are exposed to ultraviolet radiations because the radiant energy excites the solution which emits that particular colour known as

fluorescence. Hence the fluorescence analysis of the successive extracts and the powdered drug of *Chirayita* treated with different chemical reagent was done and different change in the colour so appeared was observed and noted. The details are presented in table-5 & 6.

IR Spectral Study of the Drug

Novel IR spectral study of the alcoholic extract of the drug was done by running the alcoholic extract in the IR range (3500-490 cm⁻¹) of the electromagnetic spectra and major characteristic peaks were noted table-7.

Thin Layer Chromatographic Profile

Thin layer chromatographic analysis of the ethanolic extract of *S. chirayita* was carried out using Benzene: methanol: acetic acid (45: 8: 4) as solvent system. R_f values were calculated after the development of chromatogram. The R_f values in the given solvent are used to characterize the drugs identity and purity. The results obtained are given in fig. 2; table-8.

DISCUSSION

The basic and essential requirement for the development of Unani and other traditional systems of medicine and to match it with the International standards is the standardization of drugs used in them. With the increasing use of herbal medicines worldwide and the rapid extension of the global market for its products, the safety and quality of medicinal plant materials and finished products have become a major concern for health authorities and pharmaceutical industries. From the time of collection of a drug to its storage and upto the production of medicine, chances of deterioration in quality are quite frequent, resulting in the decline of the efficacy of drug. To overcome this problem of Unani drug, it is almost inevitable to standardize the drug for their rational therapeutic use. A disease cannot be managed comprehensively until the delivery of genuine drug is ensured. Correct identification and quality assurance of the raw material is, therefore an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy. Physico-chemical and phyto-chemical studies are of prime importance in Quality Control of Unani

medicine. As the efficacy of drug mainly depends upon its physical and chemical properties therefore, their determination is necessary for the authenticity of a drug before studying any medicinal property. It is also more important, because it helps in characterization of constituents or group of constituents that frequently lead to establish the structure-activity relationship and the likely mechanism of action of the drug. Phyto-chemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying conditions. A little deviation from the normal in terms of quality and quantity of the constituents may alter the effect of drug. Apart from the degradation in the quality of the drugs that occurs due to above conditions, adulteration also contributes to variability. Thus, keeping in view the above consideration, this study was done with an aim to provide a fruitful data of standardization of *S. chirayita* for the future reference. Beside the organoleptic characters observed as a first hand information, chirayita was subjected to systematic physiochemical and phytochemical screening according to the WHO and Pharmacopeial guidelines.

The Parameters Studied and Their Significance in Standardization is as Follows

Ash value

It is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly it can also detect the nature of the material, which is added to the drug for the purpose of adulteration. Hence, determination of the ash value provides a criterion for judging the identity and purity of the drug.

Solubility

Alcohol and water soluble contents determination is an index of purity. As alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. Different percentage of alcohol (v/v in water) will vary the alcohol soluble extractives, where as the drug obtained from different sources may produce different extractive values, extracted in the same concentration of alcohol.

Moisture content

It not only gives an idea regarding the adulteration but also satisfy the basic consideration that accurate scientific works where the drug is to be sold is within guaranteed assay. So, the percentage of active constituents must be calculated on the basis of moisture free drug.

Loss of weight on drying

It indicates the loss of volatile substances along with the water.

pH values

It indicates that drug will get ionized in stomach and will be absorbed in intestine, as the drug in the opposite pH are unionized and absorbed rapidly from the stomach/ intestine accordingly.

Extractive values

It is the amount of the extract that the drug yields in a solvent; it is often an approximate measure of the amount of certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one.

Crude fibre content

Crude fibre content is of considerable importance for examining the certain drugs and particularly of species which are adulterated with the waste or refused material of the same drug and species.

Alkaloid estimation

It is one of the most physiological active compounds present in plants, most extensively investigated compounds among the secondary metabolites because of their therapeutic importance; there is specific percentage of alkaloids in respective plants

Qualitative analysis of the powder drug

Therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs and the lower percentage of chemical constituents may cause lesser therapeutic values of the drugs and therefore, they are considered as low standard drugs.

Fluorescence analysis of successive extracts

Florescence analysis under UV light is sometime very characteristic for a drug. As many drugs and

the constituents present in the drug emit specific colour when they are exposed to ultraviolet radiations because the radiant energy excites the solution which emits that particular colour known as fluorescence.

IR Spectral study

(IR-Spectra) are also helpful for the identification of the chemical constituents present in the drug. As the scanning of the drug in any solvent under infra red region by increasing the wavelength yields a graph with different peaks and troughs, where many of the peaks in particular region specify about a particular chemical constituent present in the drug.

Thin layer chromatography

It is one of the important parameter used for detecting the adulteration for judging the quality of drugs. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the number of spots. On the other hand the exhausted or deteriorated drugs may lose the component and the number of spots appeared might be less.

CONCLUSION

Table 1: Organoleptic Characters of powder of *Swertia chirayita* Linn.

S. No.	Parameter	Appearance
1.	Colour	Green
2.	Smell	Bitter
3.	Taste	Extremely bitter

Table 2: Physicochemical Analysis of *Swertia chirayita* Linn.

S. No.	Physicochemical Parameter	Results Mean±S.E.M. (S.D.)
1.	Moisture Content	
	Loss of Weight on Drying	8.69±0.01(0.02)
	Toulene Distillation Method	8.37±0.01(0.02)
2.	Ash Value (%)	
	Total Ash	2.40±0.00 (0.01)
	Acid Insoluble Ash	0.49±0.00 (0.01)
	Water Soluble Ash	3.12±0.00 (0.01)
	Sulphated Ash	0.86±0.01 (0.04)
3.	pH Values (%)	
	pH at 1%	5.49±0.00(0.01)
	pH at 10%	5.03±0.01(0.02)
4.	Bulk Density (gm/ml)	0.67±0.01(0.02)
5.	Melting Range	80-90 ⁰ C
6.	Solubility (%)	
	Alcohol Soluble extractive	3.86±0.40(0.70)
	Water Soluble extractive	5.89±0.32(0.56)

The basic and essential requirement for the development of Unani and other traditional systems of medicine and to match it with the International standards is the standardization of drugs used in them. Standardization is an integral part for any study, when we are exploring any biological activity of any drug, we should first make drug authentic according to the Pharmacopoeia. It is therefore necessary to work out physicochemical standards of unani drugs. It is concluded that now a days, many of the medicinal plants available in the market have ambiguous identification along with adulteration and contamination. The physicochemical evaluation of the powder drug reveals the standard parameters for the quality and purity of herbal drug and also gives information regarding the authenticity of crude drug.

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Table 3: Phyto-chemical Analysis of *Swertia chirayita* Linn.

S.No.	Physicochemical Parameter	Results Mean \pm S.E.M. (S.D.)
1.	Crude Fibre Content	4.80 \pm 0.07(0.04)
2.	Total Alkaloid Estimation	11.53 \pm 0.15(0.08)
3.	Extractive values in different organic solvent	
	Petroleum ether (60-80 ⁰)	0.92 \pm 0.02 (0.04)
	Diethyl Ether	0.63 \pm 0.02 (0.05)
	Chloroform	4.12 \pm 0.02 (0.04)
	Alcohol	4.35 \pm 0.11 (0.20)
	Aqueous	19.14 \pm 0.11 (0.20)

Table 4: Qualitative Analysis of the Phytochemicals of *S. chirayita* Linn.

S. No.	Chemical Constituents	Test Reagents	Chirayita
1.	Alkaloids	Dragendorff's Reagent	+ve
		Wagner's reagent	+ve
		Mayer's reagent	+ve
2.	Carbohydrates	Molish Test	+ve
		Fehling Test	+ve
		Benedict Test	+ve
3.	Flavonoids	Mg Ribbon and dil. Hcl	+ve
4.	Glycosides	NaOH Test	+ve
5.	Tannins/Phenols	Ferric Chloride Test	+ve
		Liebermann's test	+ve
		Lead Acetate test	+ve
6.	Proteins	Xanthoproteic test	-ve
		Biuret test	+ve
7.	Starch	Iodine Test	-ve
8.	Saponins	Frothing with NaHCO ₃	+ve
9.	Steroids/Terpenes	Salkowski Reaction	+ve
10.	Amino acids	Ninhydrin Solution	+ve
11.	Resins	Acetic anhydride test	+ve

Indications: '-ve' Absence and '+ve' Presence of constituents

Table 5: Fluorescence Analysis of *Swertia chirayita* Linn.

S. No.	Powdered drug (P.D)	Day Light	UV Short	UV Long
1.	P.D + Con. HNO ₃	Light Yellow	Green	Black
2.	P.D + Con. Hcl	Brown	Green	Dark Green
3.	P.D + Con. H ₂ SO ₄	Black	Black	Black
4.	P.D + Iodine solution (5%) in alcohol.	Dark Red	Dark Green	Black
5.	P.D + Glacial Acetic acid	Brownish Gr.	Green	Black
6.	P.D + Glacial Acetic acid + HNO ₃	Bright Yellow	Green	Green
7.	P.D + NaOH Solution (10%)	Brown	Green	Black
8.	P.D + 10%NaOH + Conc ⁿ HNO ₃	Light Brown	Green	Dark Green
9.	P.D + dilute HNO ₃	Light Brown	Brownish Gr.	Black
10.	P.D + dilute H ₂ SO ₄	Light Brown	Dark Green	Black
11.	P.D + dilute Hcl	Light Green	Green	Black
12.	P.D + Drangendorff reagent	Reddish Br.	Dark Green	Black
13.	P.D + Wagner's reagent	Green	Green	Dark Green
14.	P.D + Benedict's reagent	Dark Red	Green	Green
15.	P.D + Fehling reagent	Dark Green	Green	Dark Green
16.	P.D + KOH (10%) methanolic	Light Yellow	Dark Green	Dark Green
17.	P.D + CuSO ₄ (5%)	Green	Light Green	Dark Green
18.	P.D + Ninhydrin (2%) in acetone	Light Green	Green	Black
19.	P.D + Picric acid	Yellow	Light Green	Dark Green
20.	P.D + Lead Acetate (5%)	Green	Light Green	Dark Green

Table 6: Fluorescence Analysis of the successive extracts of *Swertia chirayita* Linn.

Extracts	Day Light	UV Long	UV Short
Petroleum ether	Dark Green	Light Green	Black
Diethyl ether	Green	Light Green	Dark Green
Chloroform	Dark Green	Dark Green	Black
Alcohol	Dark Green	Green	Brown
Aqueous	Brown	Dark Green	Dark Brown

Table 7: IR Spectral Study of *Swertia chirayita* Linn.

Test Drug	IR, ν (cm ⁻¹)
Chirayita (<i>S.chirayita</i> Linn.)	3448.93, 2941.47,

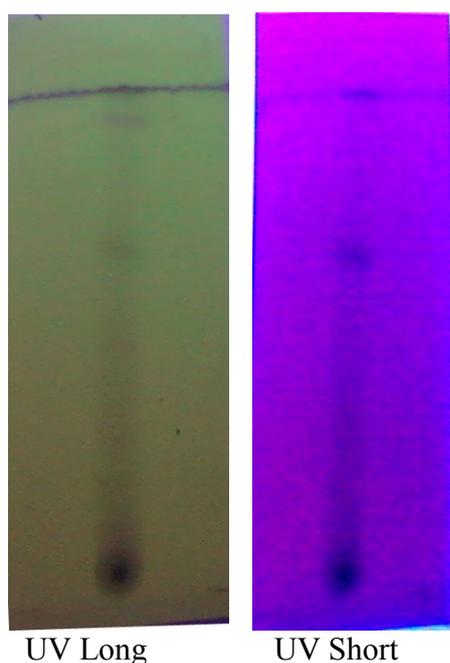
Table 8: Thin Layer Chromatography of *Swertia chirayita* Linn.

Extract	Solvent System	Treatment	Visualizing Agent	No. of Spots	R _f value
Petroleum ether	Benzene :Chloroform (3:1)	I ₂ vapour	Day Light	6	1.1, 1.6, 2.4,4.2,5.1, 8.5
			UV Long	6	1.1, 1.6, 2.4,4.2,5.1, 8.5
			UV Short	1	2.0(Br)
	Chloroform :Methanol (93:7)	”	Day Light	2	0.18,0.63
			UV Long	2	0.18,0.63
			UV Short	1	0.5(G)
	Petroleum ether : Ether (6:4)	”	Day Light	4	0.12,0.25,0.37,0.62
			UV Long	4	0.12,0.25,0.37,0.62
			UV Short	1	0.36(Fl. Bl)
Chloroform	Chloroform :Methanol	I ₂ vapour	Day Light	2	2.2, 4.2
			UV Long	2	2.2, 4.2
			UV Short	1	2.5(G)
	Ethyl Acetate :Methanol (9:1)	”	Day Light	4	0.12, 0.38, 0.84, 0.92
			UV Long	4	0.12, 0.38, 0.84, 0.92
			UV Short	5	0.15(Br),0.38(L.Br),0.76(Br),0.84(D.Br),0.92(G)
	Benzene :Ethyl acetate (9:1)	”	Day Light	5	0.02,0.11,0.14,0.78,0.85
			UV Long	5	0.02, 0.17,0.78,0.85,0.88
			UV Short	1	0.78(F.Gr)
Alcohol	Toulene :Ethyl acetate: Formic acid (5:4:1)	I ₂ vapour	Day Light	6	3.6,4.8,5.7,6.9,8.2,9.6
			UV Long	4	2.4,3.6,4.8,5.7
			UV Short	1	3.6(G)
	Benzene: Ethyl acetate (9:1)	”	Day Light	1	0.13
			UV Long	1	0.13
			UV Short	1	0.13(B)
	Methanol: Chlo-roform (1:9)	”	Day Light	5	0.08,0.4,0.5,0.53,0.91
			UV Long	5	0.08,0.4,0.5,0.53,0.91
			UV Short	4	0.4(Y),0.5 (L.Y),0.58(Y), 0.8(O)

D: Dark; L: Light; Br.: Brown; Bl: Blue; G: Green; Y: Yellow; O: Orange; B: Black; Fl.: Fluorescent



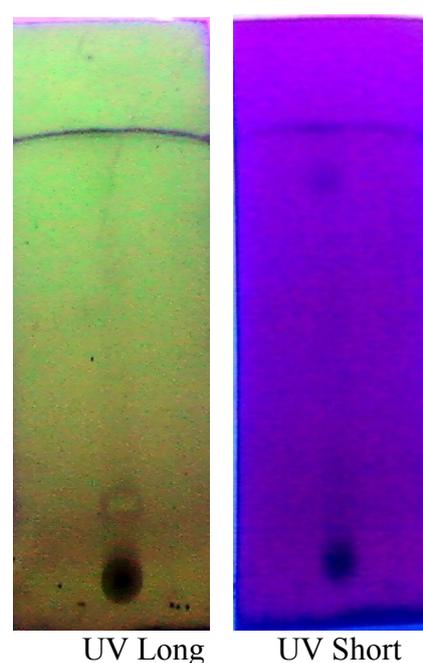
Figure 1: Sample of *Swertia chirayita* Linn.



UV Long

UV Short

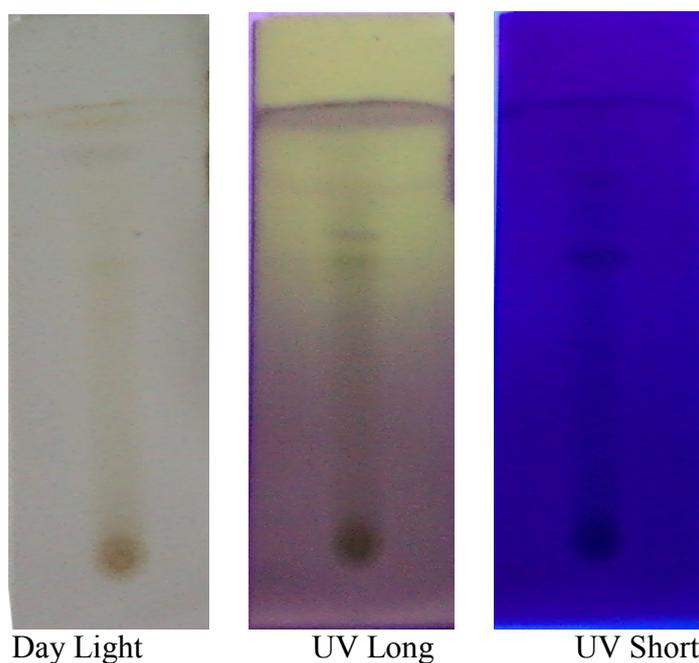
Figure 2 (a): TLC Chirayita-Petroleum Ether extract



UV Long

UV Short

Figure 2 (b): TLC Chirayita-Chloroform extract



Day Light

UV Long

UV Short

Figure 2 (c): TLC Chirayita- Ethanol extract

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