



## **Shodhana (Processing) of Gunja (*Abrus precatorius* Linn.) Seeds with Godugdha (Cow's milk); a pharmaceutical analysis**

### **Research article**

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### **Abstract**

Seeds of *Abrus precatorius* Linn. (Fabaceae), a poisonous plant drug, is being used in different Ayurvedic therapeutics, after proper processing (*Shodhana*) with some specific media. In Ayurvedic literature, media like *Godugdha* (cow's milk), *kanji* (sour gruel) *nimbu swarasa* (Lemon juice) etc. has been reported for processing of *Gunja* seeds. In the present study, the *Gunja* seeds were processed by using *Godugdha* (cow's milk) and water as media and the raw seeds were taken as control. This study reveals certain changes in different physico-chemical parameters and  $R_f$  values in HPTLC of *Godugdha shodhita Gunja* seed in comparison to the water *Shodhita* and Raw *Gunja* seed. It is observed that the colour of the media was changed to reddish ting after each *shodhana*. Changes in every physico chemical parameters confirm the effect of *shodhana* on *Gunja* seed. In HPTLC analysis, varieties of  $R_f$  value were detected in raw and *shodhita* samples indicating change in the nature of the *shodhita* drugs.

**Key words:** *Gunja*, *Abrus precatorius* Linn, purification, *shodhana*, Abrin

### **Introduction:**

*Gunja* (*Abrus precatorius* Linn.), a well-known plant of Ayurveda under *Upavisha* group (sub/semi poisonous group) (1), is being used extensively in different formulations with great therapeutic significance and is being advocated to use, in various diseases like Indralupta (alopecia), Shotha (edema), Krimi (helminthes), Kustha (skin diseases), Kandu (itching), Prameha (urinary disorders) etc. after proper

samaskar known as *shodhana* (processing or purificatory procedure) (2,3,4). The seeds of *Gunja* are often used criminally for killing cattle where the seeds are powdered and made into a paste, with which the darts or arrows are dressed (5) The concept of *Shodhana* (processing or purification) in Ayurveda is not only a process of purification or detoxification but also a process to enhance the potency and efficacy of the drug (6).

The *Gunja* seed contains number of chemical constituents like alkaloid, steroid, flavones, triterpenoides, proteins, amino acids etc., among which an albumotoxin, *abrin* (a highly toxic protein) is considered the main responsible constituent for the poisonous effect of *Abrus precatorius* Linn. With an estimated human fatal dose of 0.1-1 µg/k (7,8), and it

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is reported that boiling renders the seed harmless (5).

Effect of *shodhana* on *Gunja* seeds with cow,s milk and *kanji* reveals that the process of *shodhana* resulted in depletion of more toxic alkaloid hypaphorine and protein abrin(9) and it is also reported that *Gunja* seeds after *shodhana* with *Godugdha* shows more significant antimicrobial activity than the *raw Gunja* seeds (10). But reporting of effect on different other parameters is lacking. Hence, the present study carried out to evaluate the impact of *shodhana* of *Gunja* seeds, through *Godugdha* on physico-chemical and HPTLC profile study.

### **Material and methods:**

#### **Collection and selection of drug:**

The plant *Gunja* (*Abrus precatorius* L., Fabaceae), was identified by expert plant taxonomist with help of different flora and its mature seed (red variety) were personally collected from *Gunja* plants from surrounding place of Jamnagar, Gujarat in their natural habitat, during the month of November - January. The fully matured dry seeds were first dropped in a beaker containing water. The seeds those floated on the surface of water or found broken, fade in colour were rejected. The seeds, those settled at the bottom of the beaker were selected for purification after being dried in air and were made in to coarse powder with help of a mechanical grinder and were kept in a glass jar, considered as Raw *Gunja* Seeds (RGS), to be used for all *shodhana* purpose.

#### **Equipment for Shodhana (Purification):**

Stainless steel vessel (20 cm x 30 cm ) ; capacity of 7 L, used as Dolayantra, Stainless steel rod (28 cm), Stainless steel vessel (48 cm x 30 cm x7 cm ) ; capacity of 3L., Cotton threads 30 cm in length, Measuring mug (capacity of 1L), Muslin cloth (45 cm x 45 cm), Digital weighing machine, Digital induction cooker,

stainless steel spatula (length: 30 cm), and measuring cylinder (10 ml, 25ml).

#### **Procedure:**

In this study, *Shodhana* of *Gunja* seeds (Coarse powder) were carried out by one of the classically approved methods (1). Each *Shodhana* procedure was repeated for three times to establish the validation of the pharmaceutical processing. *Shodhana* of *Gunja* was performed by the process of *Swedana* (boiling) in *Godugdha* (12). 100 g of Raw *Gunja* seeds (RGS) were kept in a muslin cloth and made into a *pottali*. The *pottali* was hanged in a steel vessel and freshly collected *Godugdha* was filled in the vessel, up to the complete immersion of the *pottali* as per standard *Swedana* procedure (11). Boiled on an induction cooker, for six hours at 100<sup>0</sup>C, throughout the experiments. Total 7 litres of *Godugdha* was utilized for one batch throughout the process. After boiling for six hours, the seeds were taken out from *pottali* and washed with lukewarm water followed by removal of seed coat and kept on a glass plate, for the shade drying. After proper drying, the seeds were collected and stored in air tight glass container and being labeled as '*Godugdha shodhita Gunja* seed' (GSGS).

Same procedure was followed for the *Shodhana* of *Gunja* seed with Water (obtained from RO plant) and the final product was labeled as Water *shodhita Gunja* seed (WSGS).

#### **Preparation of sample:**

The Raw (RGS) and *shodhita Gunja* (Both GSGS & WSGS) seeds were powdered with mechanical grinder and passed though mesh no. 60.

#### **Physico chemical parameters:**

Assessment of the parameters such as foreign matter, moisture content, ash value, acid insoluble ash, pH with pH paper, water soluble extractive value,



alcohol soluble extractive value, foaming index and swelling index were carried out following standard procedures recommended by Ayurvedic Pharmacopoeia of India(API) (13, 14).

### HPTLC study: (15)

#### Chemicals

Percolated silica gel 60 F<sub>254</sub> TLC aluminum plates (10×10 cms, 0.2mm thick), AR grade toluene, ethyl acetate, glacial acetic acid, methanol was obtained from M/S Merck Ltd. Mumbai, India.

#### Samples for HPTLC

The extract of all three samples (RGS, GSGS & WSGS) for HPTLC, were made in same process as mentioned below.

**1. Methanolic extract** - 2 g of sample was macerated with 20ml of methanol for 24 hrs & filtered. Filtrate was concentrated to 5ml & used for spotting.

The samples were titled as Track-1, Track-2 & Track-3.

**Track-1:** Methanolic extract of *Raw Gunja* seed.

**Track-2:** Methanolic extract of *Godugdha shodhita Gunja* seed.

**Track-3:** Methanolic extract of *Water shodhita Gunja* seed.

**Mobile phase:** Toluene: Ethyl acetate: Glacial acetic acid (6.5: 3.5: 0.2) v/v/v

**Detection:** Spray with Vanilline-H<sub>2</sub>SO<sub>4</sub>.

#### Chromatographic conditions

Application mode	Camag Linomat V
Development Chamber	Camag Twin through Chamber.
Plates	Precoated Silica Gel GF254 Plates.
Chamber Saturation	30 min.
Development Time	30 min.
Development distance	7 cm.
Scanner	Camag Scanner III.
Detection	Deuterium lamp, Tungsten lamp
Data System	Win cats software

The developed plate was scanned to obtain densitogram in visible range from 600 nm to 800 nm with 100 nm interval.

### Results and Discussion

During *Shodhana* of *Gunja* with *Godugdha* and water, change in the color of both the media, from its normal colour to reddish ting, was noticed and it might be due to the removal of color containing materials from the endosperm of the seeds. The reddish cream colour powder of raw seeds turned into brownish color in case of *Godugdha shodhita Gunja* seed and Ash colour in case of water *shodhita Gunja* seed after *shodhana* (Table-1). After *shodhan* with *Godugdha* and water, 85.96% and 91.66% of purified *Gunja* seed were obtained respectively.[Table-2] It might be due to the extraction of more soluble mass from the seeds by *Godugdha* than water.

It was observed that the moisture content of *Godugdha shodhita Gunja* seed was comparatively lower than the raw and water *shodhita Gunja* seed [Table-3]. Determination of moisture content of drug is one of the important methods in plant standardization. Excess of moisture in a sample may encourage growth of microbes. Lower value of moisture content indicates less chances of microbial growth (11). Ash value was decreased in case of all samples after purification. Ash mainly contains inorganic radicles and it should be totally free from carbon particles. Lower the carbon particle in ash reduces the ash value which indicates more purity of a drug. The water soluble extractive value in *Godugdha shodhita Gunja* seed was found higher than raw water *shodhita Gunja* seed. It is being observed that all samples are acidic [Table-3]. Lower the P<sup>H</sup> value indicates more acidic in nature, which is more capable to inhibit microbes.

In HPTLC, at short UV 254nm, different spots were found in all three samples indicating presence of different



components. [Table-4] Presence of one common Rf value (0.01) in all three samples, indicates the presence of one common component to all three samples.

At long UV 366 nm, raw *Gunja* seed, *Godugdha shodhita Gunja* seed and water *shodhita Gunja* seed showed 6, 5 and 5 spots respectively. [Table-5] From the below mentioned spectral comparison (Fig-9, Fig-10, Fig-11) some same Rf value were found in case of all three samples i.e. 0.32, 0.48 & 0.96. From which it can be narrated that the presence of same component is possible in case of all three samples.

### Conclusion:

From this study, it is concluded that *shodhana* alters the physicochemical parameters of *Gunja* seeds and also the Rf value of the sample in HPTLC. Numbers of spots were decreased under both 254nm and 366nm after *shodhana*, indicating denaturation of some component after *shodhana*.

### Reference:

1. Pranacharja Shri Sadananda Sharma, Pandit Kasinathshastrina. Rasatarangini. Delhi; Motilal Banarasidas; 2009. 727-733p.
2. Gogte VM. Ayurvedic Pharmacology & Therapeutic Uses of Medicinal Plants. 1st edition. Mumbai; Bharatiya Vidya Bhavan; 2000, 345-347p.
3. Review on Indian plants. Indian council of medical research. New Delhi; 2004. 24p.
4. Malati G Chauhan and A P G Pillai. Microscopic profile of Drugs used in Indian Systems of Medicine. Vol-3. Seed drugs, Part-1. 2011. 1p.
5. Kritikar K.R & Basu B.D. Indian medicinal plants. Vol-1. DehraDun; International book distributors; 766p.
6. Shastri JLN. Dravyaguna Vijnana. 1<sup>st</sup> edition. Vol.I. Varanasi; Choukhamba Orientalia; 2009. 320p.
7. Parikh C.K. Parikh's Test book of Medical Jurisprudence Forensic Medicine and Toxicology. Sixth edition. Darya Ganj, New Delhi-110002(India); CBS Publishers & Distributors. 4596/1A, 11. 2007. 9.31-11.16p.
8. The wealth of India. Raw materials. Vol-I: A. New Delhi; Revised version.council of scientific & Industrial research; 2003. 18-20p.
9. Debnath Singh Gautam, R. Banerji, S.Malhotra. Effect of *shodhana* on the toxicity of *Abrus Precatorius*. Ancient science of life. October 1998; vol.No 18 (2).
10. Comparative study: Antimicrobial activity of *Ashodhita* and *Shodhita Shwet Gunja beej (Abrus precatorius* Linn.). Nashik; Maharashtra University of Health Sciences, 2006-2007.
11. Lohar D.R. Protocol for testing, Ayurvedic, Siddha, Unani medicines. Ghaziaba; Government of India, Department of Ayush, Ministry of Health & Family Welfare, Pharmacopoeial laboratory for Indian medicines, 30<sup>th</sup> March 2007.
12. Ayurvedic Pharmacopoeia of India (API). First edition. Part-II, Vol-II, Appendices-2. New Delhi; Government of India, Ministry of Health and Family Welfare, Department of AYUSH; 2008. 159-161p.
13. Anonymous, Planner Chromatography, Modern Thin layer Chromatography. Switzerland; 1999. 2-16p.

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**Table 1: Organoleptic characters of raw, Godugdha and water shodhita Gunja seed powder**

SL.NO	SAMPLE	COLOUR	ODOUR	TASTE	APPEARENCE
1	RGS	Reddish cream	Typical	Bitter	Smooth and shiny
2	GSGS	Brownish after drying	Characteristic of milk	Sweetish bitter	Dull
5	WSGS	Ash color	Typical	Bitter	Dull

RGS= Raw *gunja* seed; GSGS= *Godugdha shodhita Gunja* seed; WSGS= Water *shodhita Gunja* seed

**Table 2. Effect of Shodhana on yield of final product after shodhana with Godugdha and water.**

SAMPLE	INITIAL QUANTITY(g)	FINAL WEIGHT (Avg.)(g) of three batches	PERCENTAGE OF WEIGHT LOSS(%)
GSGS	100	85.96	14.04%
WSGS	100	91.66	8.34%

GSGS= *Godugdha* (Cow's milk) *shodhita Gunja* seed; WSGS= Water *shodhita Gunja* seed

**Table 3. Physico-chemical parameter of raw and shodhita Gunja seeds**

Test Parameters	Sample:1 (RGS)	Sample:2 (GSGS)	Sample:3 (WSGS)
Description	Outer coat red with black spot	Brownish after drying	Ash colour
Foreign matter	Nil	Nil	Nil
Moisture content	9.5% w/w	9.025%	9.49%
Ash value	4.944% w/w	4.644%	4.096%
Acid insoluble ash	1.5% w/	0.34%	0.54%
P <sup>H</sup> (p <sup>H</sup> paper)	5.5	5.5	5.5
Water soluble extractive value	10.35% w/v	11.38%	6.087%
Alcohol soluble extractive value	1.5%	0.69%	0.39%
Foaming index	<100	<100	<100
Swelling index	3 ml	4.5ml	3.5ml

RGS= Raw *gunja* seed; GSGS= *Godugdha shodhita Gunja* seed; WSGS= Water *shodhita Gunja* seed

**Table 4:- R<sub>f</sub> value in Short UV 254 nm of the methanolic extract of all three samples**

SL NO	SAMPLE	NO. OF SPOT	R <sub>F</sub> VALUE
1	RGS	8	0.01, 0.04, 0.11, 0.20, 0.24, 0.27, 0.46, 0.90
2	GSGS	6	0.01, 0.28, 0.39, 0.46, 0.60, 0.69
3	WSGS	4	0.01, 0.26, 0.88, 0.91

RGS= Raw *gunja* seed; GSGS= *Godugdha shodhita Gunja* seed; WSGS= Water *shodhita Gunja* seed

**Table 5:-R<sub>f</sub> value in long UV 366nm of the methanolic extract of all three samples**

SL NO	SAMPLE	NO. OF SPOT	R <sub>F</sub> VALUE
1	RGS	6	0.01, 0.05, 0.08, 0.25, 0.41, 0.91
2	GSGS	5	0.00, 0.18, 0.38, 0.46, 0.92.
3	WSGS	5	0.01, 0.40, 0.47, 0.87, 0.93

RGS= Raw *gunja* seed; GSGS= *Godugdha shodhita Gunja* seed; WSGS= Water *shodhita Gunja* seed

**HPTLC profile:-**

Short UV 254 nm	Long UV 366 nm	After spraying
Track-1 HPTLC for Methanolic extract of <i>Raw Gunja</i> seed.		
Track-2 HPTLC for Methanolic extract of <i>Godugdha shodhita Gunja</i> seed.		
Track-3 HPTLC for Methanolic extract of water <i>shodhita Gunja</i> seed.		

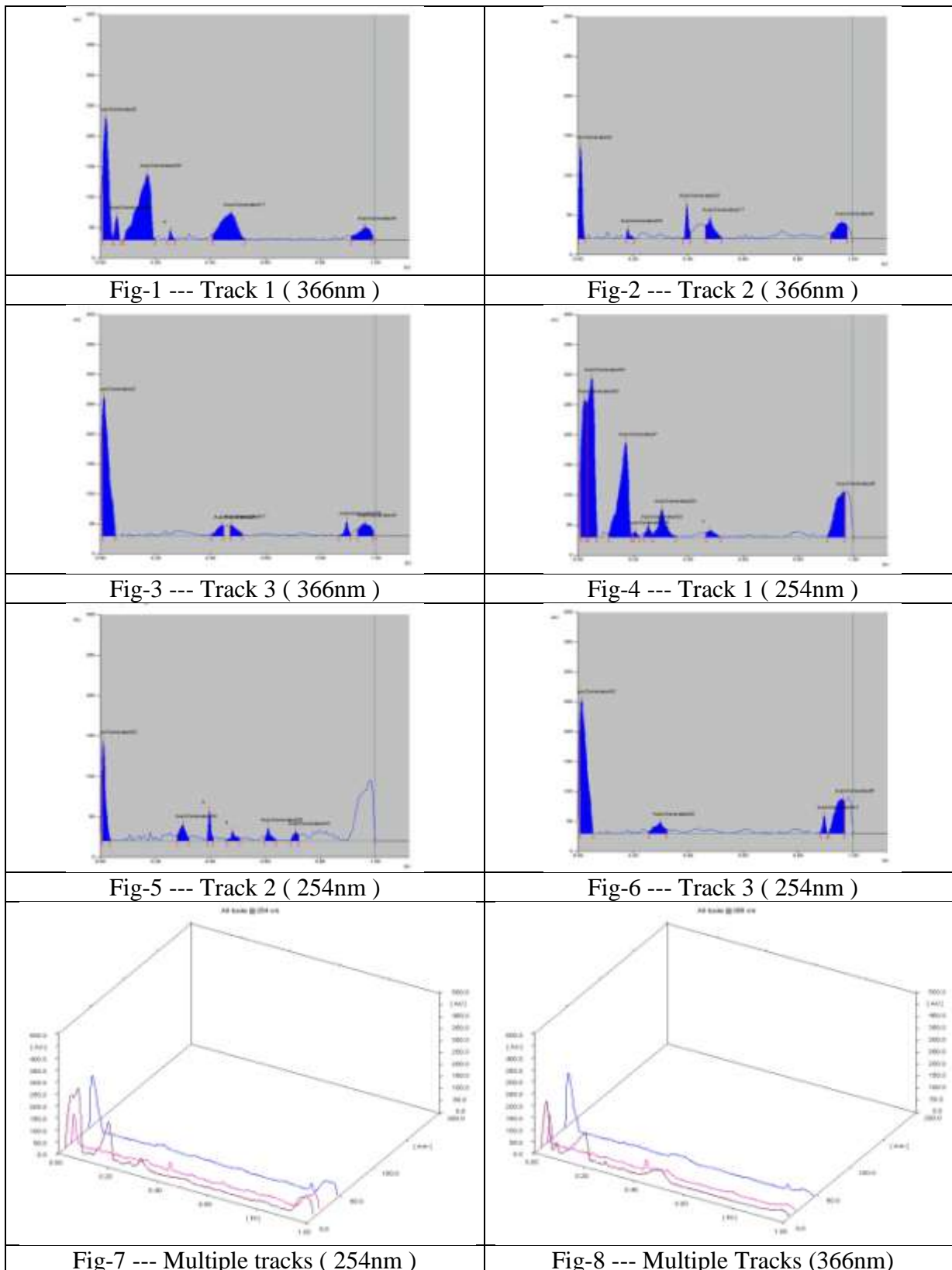


### Densitogram of Standard (visible Spectrum Comparison)

Track-1 HPTLC for Methanolic extract of *Raw Gunja* seed.

Track-2 HPTLC for Methanolic extract of *Godugdha shodhita Gunja* seed.

Track-3 HPTLC for Methanolic extract of water *shodhita Gunja* seed



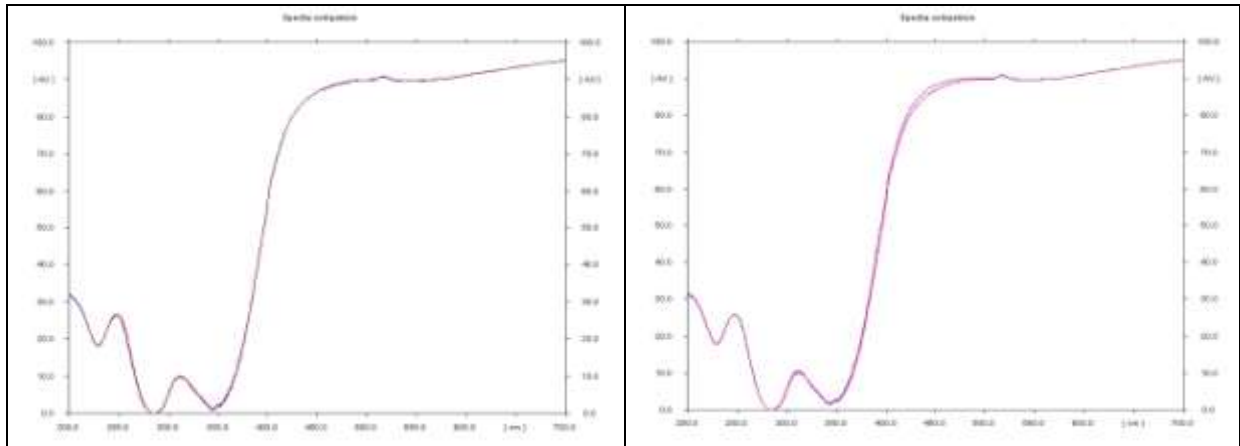


Fig-9 --- U.V.Spectral comparison Rf 0.32  
T-1,2,3

Fig-10 --- U.V.Spectral comparison Rf 0.48  
T-1,2,3

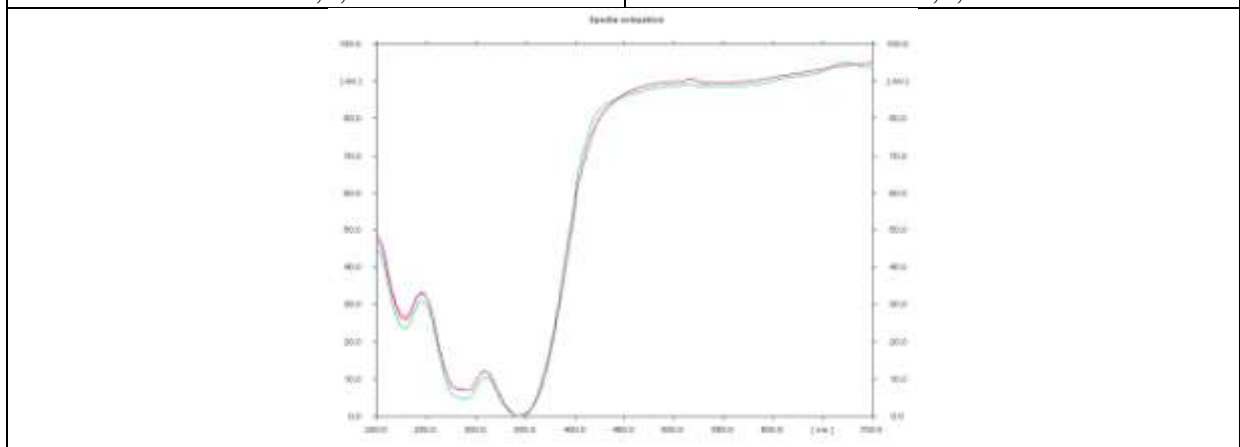


Fig-11 --- U.V.Spectral comparison Rf 0.96 T-1,2,3

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