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Access this article online
Website: www.ayujournal.org
DOI: 10.4103/0974-8520.127725
Quick Response Code:

Pharmaceutical Standardisation

Comparative physico-chemical profile of *Gunja* (*Abrus precatorius* Linn.) seeds processed through water and *Nimbu Swarasa* (lemon juice)

Sudipta Roy, Rabinarayan Acharya[1], Vinay J. Shukla[2]

Ph.D. Scholar, [1]Professor, Department of Dravyaguna, [2]Head, Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Gunja (*Abrus precatorius* Linn.), known as Indian liquorice, is reputed as one of the world's most deadly but most beautiful seed belonging to the family Fabaceae, characterised under the *Upavisha* (semi-poisonous drugs) and used extensively in various Ayurvedic formulations with great therapeutic significance. Ayurveda recommended the administration of *Gunja* only after proper *Shodhana* (purification procedures) in different media such as *Godugdha* (cow's milk), *Kanji* (sour gruel), etc., Apart from the classical methods, some traditional practitioners use *Nimbu Swarasa* for the *Shodhana* of *Gunja* seeds. In this study, an attempt has been made to carry out *Shodhana* of *Gunja* seeds using *Nimbu Swarasa* and water. This study revealed differences in physico-chemical parameters of purified samples, in comparison to raw drugs.

Key words: Abrin, *Abrus Precatorius*, *Gunja*, *Nimbu Swarasa*, *Shodhana*

Introduction

Gunja, one of the poisonous plants reported in ancient scriptures of Ayurveda, comes under *Upavisha* category.[1] *Gunja* is used in treating various diseases such as *Indralupta* (alopecia), *Shotha* (edema), *Krimi* (helminthes), *Kustha* (skin diseases), *Kandu* (itching), *Prameha* (urinary disorders), etc., after being passed through specific *Shodhana*.[2-4] The seeds are often used criminally for killing cattle and it is reported that boiling renders the seed harmless.[5]

It is cited in the classics that *Visha* (poison) becomes *Amrita* (nectar) after logical administration[6] and the ancient physicians of Ayurveda successfully used this drug in a number of diseases after proper purification in some specific media. *Gunja* seeds contain various number of alkaloids, steroids, flavones, triterpenoides, proteins, amino acids, etc., among which albumotoxin and abrin are considered as the main responsible constituents for its poisonous effect. with an estimated human fatal dose of 0.1-1 µg/k.[7,8] *Gunja* has been reported for its antitumor,[9] anticancer,[3] antispermatogenic,[10] antifertility,[3] CNS (Central nurvous system) depressant and analgesic activity in rat,[3] in treatment of ulcer and skin affections,[8] antidiarrheal and antihelminthic[8] activities.

While going through the literatures, it can be understood that specific medium is used for *Shodhana* of particular substances and has a three-way effect on the drug, i.e. purification, detoxification, and potentiation.[11] Studies have also shown that the toxic substances present in the plant drug are transferred into the media during the *Shodhana* process rendering the drug nontoxic.[12] Specific *Shodhana* procedures have been prescribed for purification of *Gunja* seeds.[13] Studies showed that *Gunja* when purified with cow's milk or *Kanji*, resulted in depletion of toxic alkaloid hypaphorine and abrin.[14] But, studies on effect of *Shodhana* with *Nimbu Swarasa* on *Gunja* seed.

Considering this, the study has been planned to evaluate the impact of *Shodhana* through *Nimbu Swarasa* and water on *Gunja* seeds.

Address for correspondence: Prof. Rabinarayan Acharya, Department of Dravyaguna, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar - 361 008, Gujarat, India.
E-mail: drrnacharya@gmail.com

Materials and Methods

Collection of drug

The plant *Gunja* was identified by expert plant taxonomist with the help of different flora and its mature seed (red variety) was personally collected from surrounding places of Jamnagar, Gujarat in their natural habitat, during the month of November 2011-January 2012.

Selection of seed
Fully matured dry seeds were first kept in a beaker containing water. The seeds those floated on the surface of water or found broken and fade in colour, were rejected. The seeds those settled at the bottom of the beaker, were selected for purification by following procedure mentioned in Vanausadhi Viseshanka.[13]

Ingredients
- *Ashuddha Gunja* seeds 300 g (100 g for each batch)
- *Nimbu* (*Citrus medica*) *Swarasa* (juice) 18 L (6 L for each batch).
- Principle: *Swedana* (Boiling).

Preparation of media
Matured fruits of *Nimbu* were collected from the local market and juice was extracted manually.

Equipment for *Shodhana*
Stainless steel vessel (20 cm × 30 cm); capacity of 7 L (used as *Dolayantra*), stainless steel rod (length 28 cm), stainless steel vessel (48 cm × 30 cm × 7 cm); capacity of 3 L, cotton threads 30 cm in length, measuring mug (capacity of 1 L), muslin cloth (45 cm × 45 cm), digital weighing machine, pyrometer, digital induction cooker, stainless steel knife (blade: 15 cm × 2 cm), frying pan (diameter: 20 cm), stainless steel spatula (length: 30 cm), and measuring cylinder (10 ml, 25 ml).

Procedure
Hundred grams of RGS were kept in a muslin cloth and made into a *Pottali*, which was immersed in a steel vessel that is filled with *Nimbu Swarasa*.[12] Then the assembly was boiled on an induction cooker for 3 h at 100°C throughout the experiment. Totally, 6 L of *Nimbu Swarasa* was utilized for one batch throughout the process. After boiling for 3 h, the seeds were taken out from *pottali* and washed with lukewarm water. Followed by removal of seed coat manually and allowed to dry in shade by placing on a paper sheet. Same procedure was carried out for all the three batches. After proper drying, the seeds were collected and stored in air tight container and labeled as *Nimbu Swarasa Shodhita Gunja* seeds (NSGS).

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* seeds (WSGS).

Preparation of sample
The RGS and *Shodhita Gunja* (both NSGS and WSGS) seeds were powdered 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.[15,16]

HPTLC Study

Equipment for HPTLC
A HPTLC system equipped with a sample applicator Linomat V sample applicator (CAMAG, 4132 Muttenz, Switzerland) was used for application of samples. CAMAG Scanner III and Win cats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates.[17]

Chemicals
Precoated silica gel 60 F_{254} TLC (Thin Layer Chromatography) aluminum plates (10 × 10 cm, 0.2 mm thick), AR grade toluene, ethyl acetate, glacial acetic acid, and methanol were obtained from M/S Merck Ltd. Mumbai, India.

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

Methanolic extract: 2 g of sample was macerated with 20 ml of methanol for 24 h and filtered. Filtrate was concentrated to 5 ml and used for spotting.

The samples were titled as Track-1, Track-2, and Track-3.
Track-1: Methanolic extract of RGS
Track-2: Methanolic extract of NSGS
Track-3: Methanolic extract of WSGS
Mobile phase: Toluene: Ethyl acetate: Glacial acetic acid (6.5:3.5:0.2)
Detection: Spray with Vanillin–H_2SO_4.

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

In this study, *Shodhana* of *Gunja* seeds were carried out by traditionally approved method. 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 *Nimbu Swarasa*, for 3 h. same process was followed for *Swedana* in water (to serve as a control). Principles of *Swedana* methods are the extraction process where the solvent enters the cells resulting in the swelling of tissues making easy escape of the soluble constituent. The rate of extraction depends mainly on the temperature and concentration gradient across the cell membrane. Rising of temperature increases the concentration gradient across the cell membrane, thereby increase mass transfer of active principles from solid material to the solvent.[18]

During *Shodhana* of *Gunja* in *Nimbu Swarasa* and water, change in colour in *Gunja* seed m powder media was noticed and it might be due to the removal of colour containing materials from the endosperm of the seeds. The reddish cream colour powder of raw seeds turned into creamish yellow in colour in case of NSGS and ash colour in case of WSGS after *Shodhana*

[Table 1]. It was observed that 83.9% and 91.66% of purified *Gunja* were obtained after purification in *Nimbu Swarasa* and water respectively [Table 2]. It might be due to the extraction of more soluble mass from the seeds by *Nimbu Swarasa* than water.

The moisture content of NSGS was comparatively lower than the raw and WSGS. Excess of moisture in a sample may encourage the growth of microbes. Lower value of moisture content indicates less chances of microbial growth.[16] Ash value was decreased in both the samples after purification. Ash values in *Nimbu Swarasa* purified seeds were comparatively less than that of the water *Shodhita* and RGS. Ash mainly contains inorganic radicals 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 NSGS was found lower than raw sample but higher than that of the WSGS. It is being observed that all samples are having acidic pH. The pH value was found comparatively lower in NSGS (5.0) than the other two samples [Table 3]. According to some experts, acidic pH indicates *Ushnavirya*.[18]

In HPTLC, at short UV 254 nm, huge number of different spots was found in all three samples, which indicates the presence of different components [Table 4]. Presence of one R_f value (0.01) was found in all three samples, which indicates the presence of one common component in all three samples [Figures 1, 7-10].

At long UV 366 nm, RGS, NSGS, and WSGS showed 6, 11, and 5 spots, respectively. One similar R_f value, (0.01) was detected in all three samples, indicating the presence of one similar compound in all three samples [Figures 2, 4-6 and 11]. Maximum numbers of spots were found in case of NSGS (11 spots), indicating the presence of more components in NSGS than the other two samples (RGS and WSGS) [Table 5]. From the spectral comparison [Figures 12-14], same R_f values were found in case of all three samples, i.e. 0.3, 0.48, and 0.92. From which it can be narrated that the presence of same component is possible in case of all three samples. After spraying with vanillin-H2SO4, RGS, NSGS and WSGS showed 2 (0.71, 0.94), 5 (0.15, 0.60, 0.69, 0.85, 0.94) and 3 (0.60, 0.69, 0.94) spots, respectively [Figure 3].

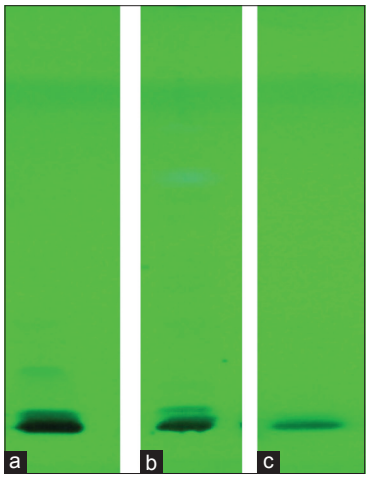

Figure 1: Short UV 254 nm. (a) Track-1: HPTLC for Methanolic extract of raw *Gunja* seed. (b) Track-2: HPTLC for methanolic extract of *Nimbu Swarasa Shodhita Gunja* seed. (c) Track-3: HPTLC for methanolic extract of water *Shodhita Gunja* seed

Table 1: Organoleptic characters of raw, *Nimbu Shodhita* and water *Shodhita Gunja* seed powder

Sample	Color	Odor	Taste	Appearance
RGS	Reddish cream	Typical	Bitter	Smooth and shiny
NSGS	Creamish yellow	Characteristic of *Nimbu Swarasa*	Sour with bitter	Dull
WSGS	Ash color	Typical	Bitter	Dull

RGS: Raw *Gunja* seed, NSGS: *Nimbu Swarasa Shodhita Gunja* seed, WSGS: Water *Shodhita Gunja* seed

Table 2: Effect of *Shodhana* on yield of final product after *Shodhana* with *Nimbu Swarasa* (lemon juice) and water

Sample	Initial quantity (g)	Final weight (g)	% of loss
NSGS	100	83.9	16.1
WSGS	100	91.66	8.34

NSGS: *Nimbu Swarasa*, *Shodhita Gunja* seed, WSGS: Water *Shodhita Gunja* seed

Table 3: Physico-chemical parameters of raw and *Shodhita Gunja* seed

Test parameters	RGS	NSGS	WSGS
Foreign matter	Nil	Nil	Nil
Moisture content (%)	9.5	7.135	9.49
Ash value (%)	4.944	2.372	4.096
Acid insoluble ash (%)	1.5	0.29	0.54
pH (pH paper)	5.5	5.0	5.5
Water soluble extractive value (%)	10.35	9.36	6.087
Alcohol soluble extractive value (%)	1.5	0.99	0.39
Foaming index	<100	<100	<100
Swelling index	3 ml	3.25 ml	3.5 ml

RGS: Raw *Gunja* seed, NSGS: *Nimbu Swarasa Shodhita Gunja* seed, WSGS: Water *Shodhita Gunja* seed

Table 4: R_f values in short UV (254 nm) of the methanolic extract of all three samples

Sample	No. of spots	R_f value
RGS	8	0.01, 0.04, 0.11,0.20, 0.24,0.27, 0.46, 0.90
NSGS	9	0.01, 0.04, 0.09, 0.24, 0.38, 0.47, 0.58, 0.65, 0.91
WSGS	4	0.01, 0.26, 0.88, 0.91

RGS: Raw *Gunja* seed, NSGS: *Nimbu Swarasa Shodhita Gunja*, WSGS: Water *Shodhita Gunja* seed

Table 5: R_f values in long (UV 366 nm) of the methanolic extract of all three samples

Sample	No. of spots	R_f value
RGS	6	0.01, 0.05, 0.08, 0.25, 0.41, 0.91
NSGS	11	0.01, 0.05, 0.09, 0.22, 0.26, 0.31, 0.38, 0.47, 0.63, 0.70, 0.84
WSGS	5	0.01, 0.40, 0.47, 0.87, 0.93

RGS: Raw *Gunja* seed, NSGS: *Nimbu Swarasa Shodhita Gunja* seed, WSGS: Water *Shodhita Gunja* seed, UV: Ultra violet

Conclusion

After *Shodhana*, changes in physico-chemical parameters of *Gunja* seeds are observed and more numbers of spots are detected under both 254 nm and 366 nm in case of NSGS, indicating the presence of more number of components

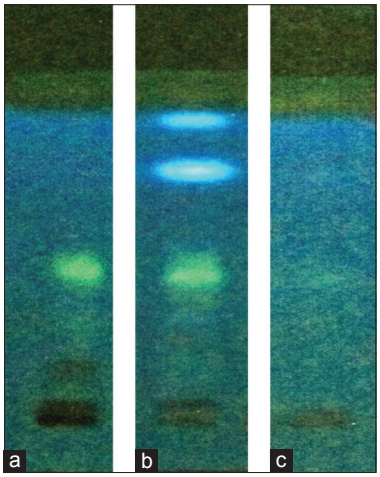

Figure 2: Long UV 366 nm. (a) Track-1: HPTLC for Methanolic extract of raw *Gunja* seed. (b) Track-2: HPTLC for methanolic extract of *Nimbu Swarasa Shodhita Gunja* seed. (c) Track-3: HPTLC for methanolic extract of water *Shodhita Gunja* seed

Figure 3: After spraying. (a) Track-1: HPTLC for Methanolic extract of raw *Gunja* seed. (b) Track-2: HPTLC for methanolic extract of *Nimbu Swarasa Shodhita Gunja* seed. (c) Track-3: HPTLC for methanolic extract of water *Shodhita Gunja* seed

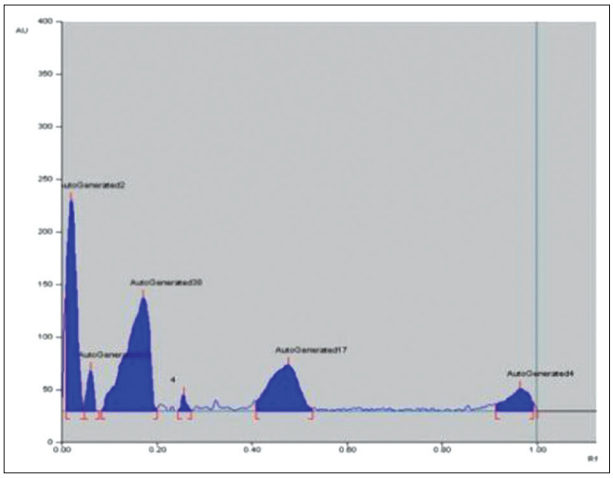

Figure 4: HPTLC for methanolic extract of raw *Gunja* seed (366 nm)

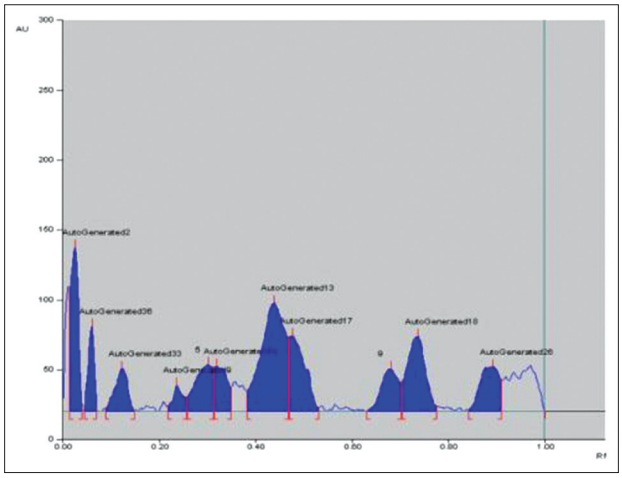

Figure 5: HPTLC for methanolic extract of *Nimbu Swarasa Shodhita Gunja* seed (366 nm)

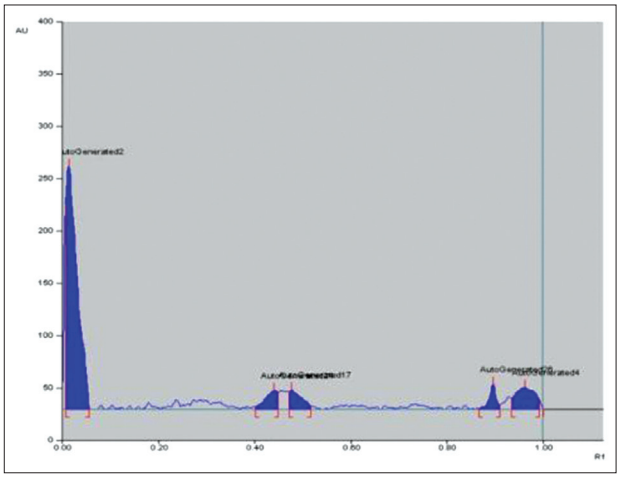

Figure 6: HPTLC for methanolic extract of water *Shodhita Gunja* seed (366 nm)

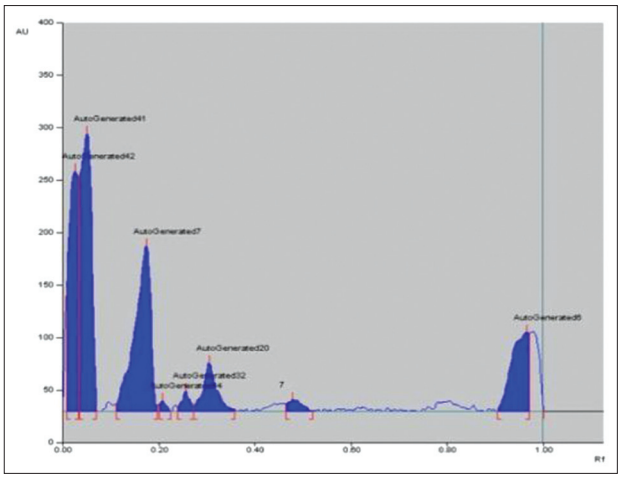

Figure 7: HPTLC for methanolic extract of raw *Gunja* seed (254 nm)

in NSGS than the other two samples (RGS and WSGS). However Qualitative estimation of these components, their utility in therapeutics need to be evaluated in further studies.

Acknowledgments

Authors are thankful to the Director, Institute for Post Graduate Teaching and Research in Ayurveda for providing

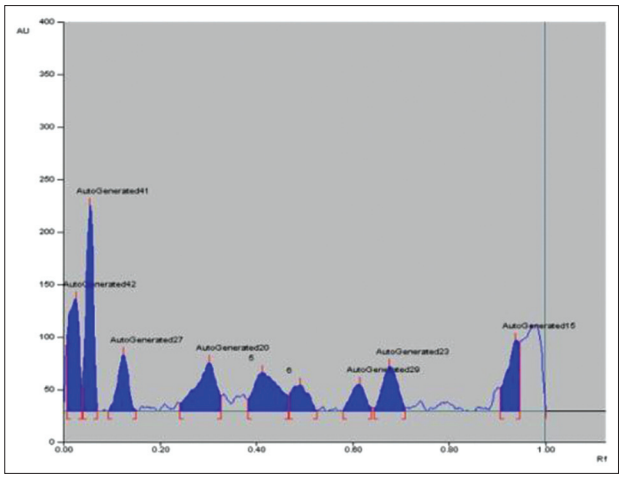

Figure 8: HPTLC for methanolic extract of *Nimbu Swarasa Shodhita Gunja* seed (254 nm)

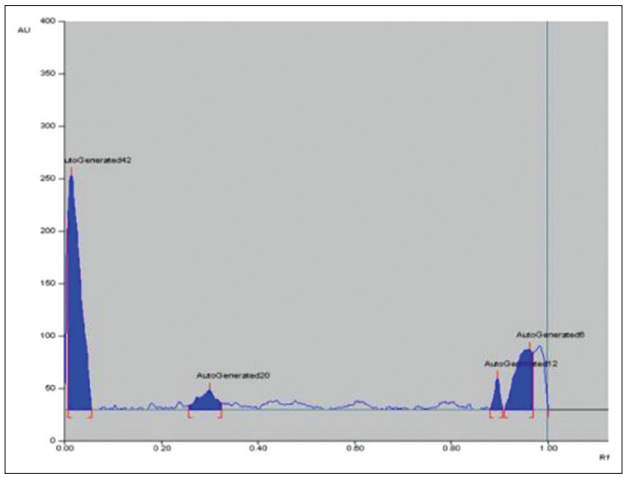

Figure 9: HPTLC for methanolic extract of water *Shodhita Gunja* seed (254 nm)

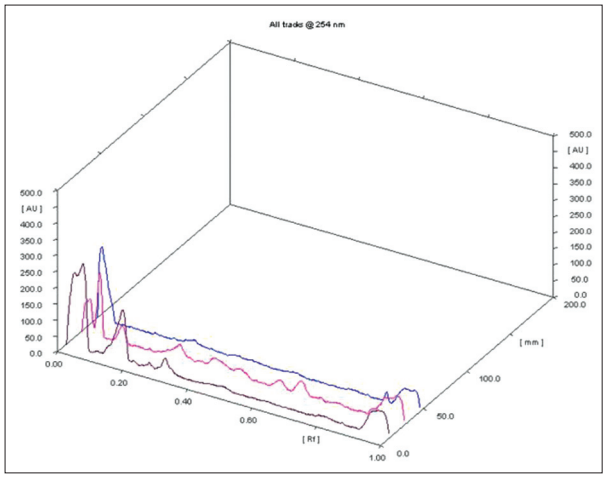

Figure 10: Multiple tracks (254 nm)

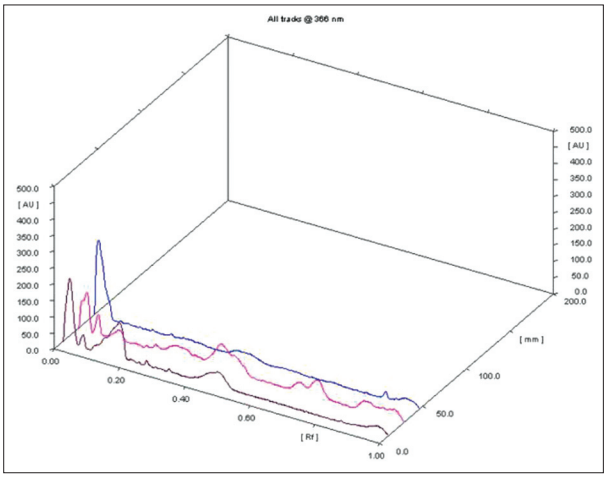

Figure 11: Multiple tracks (366 nm)

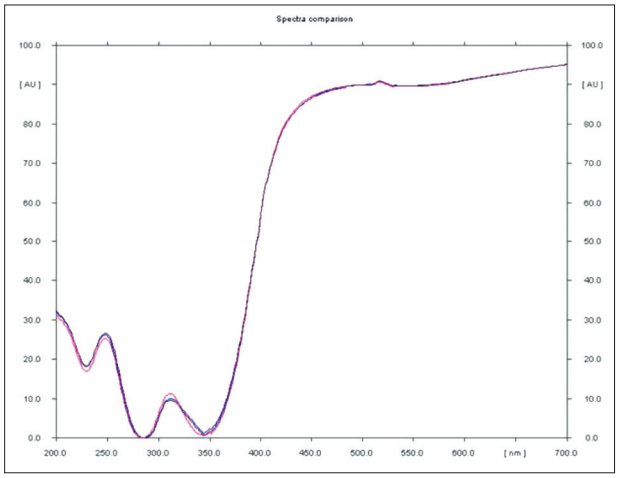

Figure 12: UV spectral comparison R_f 0.3 T-1,2,3

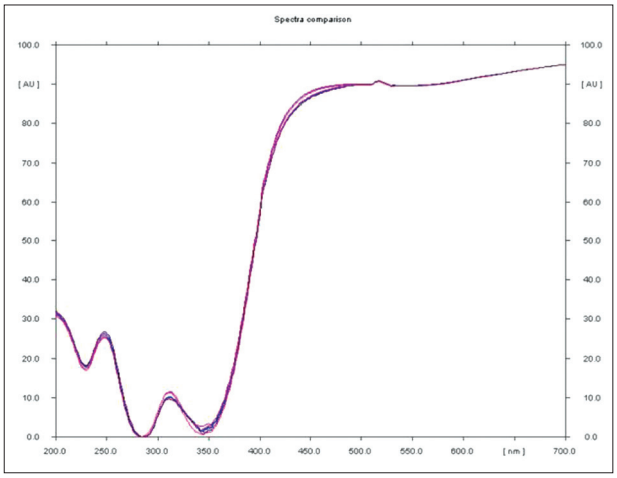

Figure 13: UV spectral comparison R_f 0.48 T-1, 2, 3

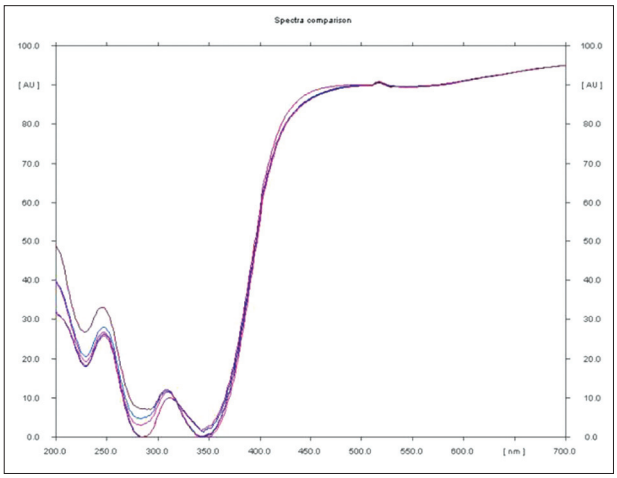

Figure 14: UV spectral comparison R_f 0.92 T-1,2,3

facilities to carry out the research work and staff of pharmaceutical laboratory for necessary help and guidance during the study.

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हिन्दी सारांश

जल एवं निम्बु स्वरस के माध्यम से शोधित गुन्जा बीज का फीजिको–केमीकल एवं क्रोमेटोग्राफीकल अध्ययन

सुदीप्ता रॉय, रबिनारायण आचार्य, विनय जे. शुक्ला

गुन्जा (इन्डियन लिकरिस, फॅबेसी कुल) उपविष श्रेणी में आने वाला एक विषाक्त वनस्पति है । इसका प्रयोग आयुर्वेद औषधियों में वर्षों से हो रहा है । आयुर्वेद में गुन्जा बीज का प्रयोग मात्र शोधन के पश्चात ही बताया गया है । शोधन के लिये गोदुग्ध, कान्जी, इत्यादि का वर्णन आयुर्वेद शास्त्र मे है । अनेक वैद्य गुन्जा बीज के लिये निम्बु स्वरस का भी प्रयोग बताते है । इस अध्ययन में गुन्जा बीज का शोधन निम्बु स्वरस एवं जल के माध्यम से किया गया है । वर्तमान अध्ययन में फीजिको–केमीकल एवं क्रोमेटोग्राफीकल स्तरों पर मानकीकरण किया गया है, जिसमे फीजिको–केमीकल परीक्षण में बहुत सारे परिवर्तन देखे गये एवं एचपीटीएलसी करने पर प्रमाणित हुआ है कि, शोधित गुन्जा मे बिन्दु संख्या अशुद्ध गुन्जा की तुलना में अधिक है जो कि शोधन के बाद गुन्जा मे आये हुए परिवर्तन को दर्शाता है ।