IJPSR (2019), Volume 10, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 June 2018; received in revised form, 15 October 2018; accepted, 10 February 2019; published 01 March 2019

ESTIMATION OF PROTEINS IN PLANTS OF FORENSIC SIGNIFICANCE (*RICINUS COMMUNIS* AND *CANNABIS SATIVA*) BY UV-SPECTROPHOTOMETRY AND GEL ELECTROPHORESIS METHOD

Astha Pandey *, Dushyant Pendarkar, Amruta D. Patel and Shalvi Agrawal

Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar - 382007, Gujarat, India.

Keywords:

Cannabis sativa, Protein estimation, Proteomics, *Ricinus communis*, SDS PAGE, UV spectrophotometry, *etc*.

Correspondence to Author:
Astha Pandey

Assistant Professor,
Institute of Forensic Science,
Gujarat Forensic Sciences University,
Gandhinagar - 382007, Gujarat, India.

E-mail: aasthapande@gmail.com

ABSTRACT: Proteins play a significant role in living beings. In animals, where proteins are involved in the body built, the formation of tissues, organs, *etc*., in plants, proteins regulate the respiration, photosynthesis, growth, and reproduction. There are some proteins which are specific to the plant or a particular species of plant. Proteomics is a multidisciplinary branch of science that deals with the study of proteins. The paper discusses some important aspect related to estimation and identification of proteins. Protein was estimated in two plants of forensic importance, *i.e.*, *Ricinus communis* and *Cannabis sativa* seeds by Ultra Violet (UV) spectrophotometry followed by isolation and characterization of proteins by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) method. A UV spectrum was taken at 520 nanometers (nm), and the protein was quantified by the Folin-Ciocalteu method. SDS-PAGE was performed according to *Laemmli et al.* High concentration of protein was found by UV spectrophotometry at 520 nm. SDS-PAGE separated the proteins according to their molecular weight ranging in size from 45 kiloDalton (kDa) to approximately 15kDa and 45kDa to 10kDa for *Ricinus communis* and *Cannabis sativa* respectively. Thus, we found in our study that both of the techniques used, *i.e.* UV spectrophotometry and SDS PAGE, proved to be indispensable in studying plant proteins and the techniques can be of great help to forensic investigators and to other laboratories concerned in dealing with plants and proteomics.

INTRODUCTION: Plant proteins are of utmost importance not only in providing health and nutritional benefits but also in the identification of the plant itself from which the protein has been derived. Excessive protein in the diet can be toxic to liver and kidneys. There are certain proteins that are poisonous like ricin from *Ricinus communis* which is a water-soluble glycoprotein and a powerful allergen.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(3).1518-23

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(3).1518-23

50-100 microgram of purified ricin powder (*i.e.*, the size of few grains of table salt) is sufficient to kill an adult human. Proteins are involved in gene expression that in turn controls the synthesis of many molecules in the plant.

In the case of *Cannabis sp*., proteins control the synthesis of tetrahydrocannabinol and other cannabinoids. Different tissues produce varying amount of proteins that ultimately is responsible for different levels of cannabinoids in different parts of the plant [1]. The finding of protein in various parts of the plant can be useful in associating the legal status of the plant or its part concerning cultivation, sale, manufacturing, and consumption under the Narcotic Drugs and Psychotropic Substances (NDPS) Act.

Beside this, proteins play a significant role in the taxonomic characterization of the plant by identification and estimation of molecular makeup through proteins, even if the whole plant is not available and only a part of it is found [2]. It helps in knowing the plant's identity and its geographical origin. Ultimately, the information from generating plant molecular databases can be used to correlate specific markers with known plant varieties; to establish forensic linkages, to make patent applications for new varieties, and to assess the degree of genetic variation found within a plant population [3]. For such purposes, characterizing proteins is useful to determine the structural, functional, and other biologically important information. In the present article, protein estimation of two forensically important plants (*Ricinus communis* and *Cannabis sativa*) has been done.

Cannabis products are the most widely trafficked drugs worldwide, accounting for 65 percent of all global seizure cases (1.65 million cases) in 2006. 5,200 metric tons of herb and 1,000 metric tons of resin were seized in 2006. Practically all countries in the world are affected by *cannabis* trafficking. Similarly, *cannabis* also remains the most widely used drug worldwide, with an estimated 166 million people have used *cannabis* in 2006, equivalent to some 4 percent of the global population aged 15-64. [4]

At the same time, especially since the end of the last century, production methods have become increasingly sophisticated, resulting in the availability in illicit markets of a wide range of *cannabis* products with widely varying levels of the main psychoactive ingredient, delta-9-tetrahydrocannabinol (THC). Most recently, there has also been a renewed debate about increasing THC content (frequently referred to as "potency") in illicit *cannabis* products [5]. All of this requires analytical data which are comparable between laboratories and over time.

Ricin is one of the most lethal plant toxins known to humans today. Ricin is produced in the seeds of castor oil plant, *Ricinus communis* [6]. 5 to 10 seeds of castor oil plant prove lethal to an adult human. The entire plant is poisonous, though seeds are most poisonous among all parts containing toxalbumin ricin in the highest amount. It is a powerful allergen (Castor Bean Allergen- CBA). Ricin is a potential chemical warfare agent often used by terrorists and militant groups for mass destruction due to its easy availability, low cost, easy absorption through the skin and there has been no vaccine available for it till date [7]. Thus, the estimation of protein in *Ricinus communis* becomes important to know the plant's identity if encountered in any criminal investigation or any other offense. Estimation of plant proteins can be done by the spectrophotometric method as well as by gel electrophoresis method.

Determining the exact quantity of proteins in a solution is very often necessary in the biochemical practice. There are many ways to measure protein concentration. In chromogenic methods, the absorbance of a colored product formed by the protein and an organic molecule is measured. Protein estimation by UV spectrophotometric method using Folin-Ciocalteu reagent is a sensitive technique in which a colored product is formed similarly to the biuret reaction, but a second reagent (Folin-Ciocalteu reagent) is used in addition to strengthening the color. The strong blue color is created by two reactions: (1) formation of the coordination bond between peptide bond nitrogens and a copper ion and (2) reduction of the Folin-Ciocalteu reagent by tyrosine (phospho-molybdic and phosphotungstic acid of the reagent reacts with phenol).

The advantages of the method include that it is quite sensitive and can detect even 1 µg of protein. Its disadvantages are that it takes rather long to carry out, is disturbed by various materials (including ammonium sulphate, glycine, and mercaptans) and that the incubation time is critical. As different proteins contain different amounts of tyrosine, the amount of colored product will also be different. As a consequence, this method is more suited to compare the concentration of solutions of the same protein than to absolute measurement.

SDS-PAGE is useful for determining the approximate molecular weight as compared to a standard mixture of proteins. The theory behind electrophoresis involves measurement of the movement of charged molecules through a medium (in this case, polyacrylamide gel) when an electric

field is generated through the medium. In general, electrophoresis can separate molecules based on size, shape, charge, and structure as these characteristics diffuse through the medium at different rates. In SDS-PAGE, sample proteins are denatured with the SDS detergent to unfold the non-polar regions and to uniformly distribute charge. Larger molecules take longer to diffuse through the gel matrix and are deposited near the top of the gel. Smaller molecules travel faster and are found closer to the bottom of the gel. Using a standard protein mixture as a reference, an unknown sample can be measured against a variety of known proteins (which should be listed with corresponding molecular weights on the standard solution) to estimate the molecular weight of the unknown protein and its subunits.

MATERIALS AND METHOD: Here we have estimated the proteins in *Ricinus communis* and *Cannabis sativa* seeds by UV-Spectrophotometric method followed by isolation and characterization of proteins by SDS-PAGE. The following protocol was followed:

Estimation of Protein by UV-Spectrophotometry:

Extraction from the Sample for UV-Spectrophotometric: Both the samples (*Ricinus* and *Cannabis* seeds) were dried and crushed to make a fine powder. The samples of *Ricinus* seeds were extracted in methanol and chloroform separately, and seeds of *Cannabis* were extracted in methanol, chloroform, cyclohexane, and petroleum-ether separately. The extracted solutions were scanned for absorption between 200-800 nm using LABINDIA UV 3000+ UV spectrophotometer under absorption mode with fast scan speed and 1.0 cm slit width.

Quantitative Analysis of Protein in *Ricinus communis* and *Cannabis sativa* Seed by Folin's Ciocalteu Method:

Protein Extraction Buffer (10 mL): 0.625 mL of 1M Tris-HCl, pH6.8 + 2 mL 10% SDS (w/v) + 1 mL 1M DTT + 1 mL 100% Glycerol + 5.375 mL distilled water.

Alkaline Copper Reagent: 50 mL 2% Na_2CO_3 in 0.1% NaOH + 0.5 mL 2% sodium-potassium tartrate + 0.5 mL 1% copper sulphate.

Folin's-Ciocalteu Reagent: Commercial Folin's reagent (2N) was diluted with an equal amount of water at the time of use.

Standard Protein Sample: Bovine serum albumin (BSA) solution- 200 µg/mL.

Extraction Procedure: 50 mg of crushed seeds of both the samples were extracted using 20 µL/mg of protein extraction buffer, vortexed and then centrifuged at 13000 rotations per minute (rpm) for 1 min. In the case of *Ricinus*, both fresh as well as dried samples were extracted for protein estimation. In the case of *Cannabis*, a diluted sample (1:100) is also prepared. Tubes containing sample were incubated for 1 hour at room temperature followed by incubation for 2 min at 100 ºC. Tubes were again centrifuged for 10 min at 13000 rpm, and the supernatant was taken into the new micro-centrifuge tube. The supernatant was centrifuged at 13000 rpm for 15 min, and the samples were stored at -30 ºC for further use.

Methodology: To the different aliquots of standard protein solutions and unknown samples, distilled water was added to make the final volume of 1.0 mL. 5.0 mL of alkaline copper reagent was added in all tubes, mixed well and incubated at room temperature for 10 min. 0.5mL of Folin's reagent was added in all the tubes and mixed well, and then tubes were incubated at room temperature for 30 min. The absorbance of each tube was estimated at 520nm. Calibration graph was plotted based on the readings.

Isolation and Characterization of Proteins by SDS-PAGE: The protocol for isolation and characterization of proteins was done by SDS-PAGE (Laemmli, 1970) [8]. 16 µL of protein sample extracted from the seeds of *Ricinus* (both fresh and dried sample) and *Cannabis* separately by following the same procedure as in case of Folin's method and 4 µL of (1X) sample buffer were combined in an Eppendorf microcentrifuge tube (1.5 mL). The sample was heated at 100 ºC for 2-10 min and then centrifuged for 1 min. The sample was introduced into the gel wells using a micropipette. The gel was run at constant voltage (190 V) until the tracking dye reached the bottom. The gel was then stained with a small amount of Coomassie brilliant blue R-250, and the glass plate

was gently agitated in the staining solution until the gel got separated from the plate. The gel was agitated on an orbital shaker overnight. After overnight staining, the staining solution was poured out and the gel was rinsed with water several times. About 50 mL of coomassie destain was added to the container containing the gel and was kept overnight on a rotary shaker, and the destaining solution was changed every hour. Eventually, the gel was observed against a white background.

RESULTS AND DISCUSSION: The qualitative tests for proteins was done on seed extract of *Ricinus communis* and *Cannabis sativa* seeds by UV spectrophotometry, gave the results as shown in **Table 1** and **Fig. 1, 3, 4, 5** and **6**. The lambda max values are by the range in which proteins absorb UV thus, confirming for the presence of proteins in all the samples.

TABLE 1: λ_{max} VALUES OF *RICINUS* AND *CANNABIS* SAMPLES IN DIFFERENT EXTRACT BY UV - SPECTROPHOTOMETRIC METHOD

S. no.	Extract	λ_{max} (nm)
1	*Ricinus* (Chloroform)	273
2	*Cannabis* (Chloroform)	272
3	*Cannabis* (Cyclohexane)	274
4	*Cannabis* (Methanol)	258
5	*Cannabis* (Petroleum Ether)	277

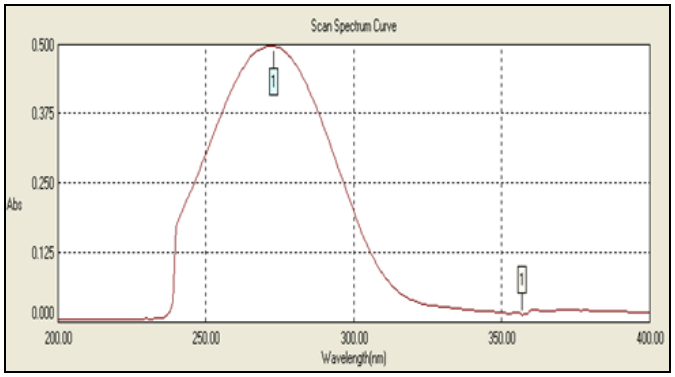

FIG. 1: λ_{MAX} PLOT OF *RICINUS* SEED PROTEIN

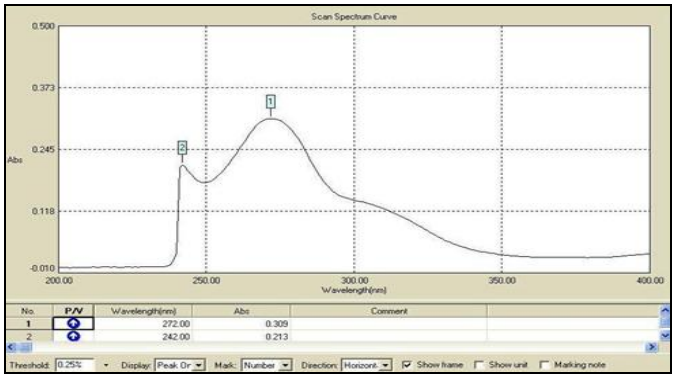

FIG. 3: ABSORBANCE SPECTRA OF *CANNABIS* CHLOROFORM EXTRACT

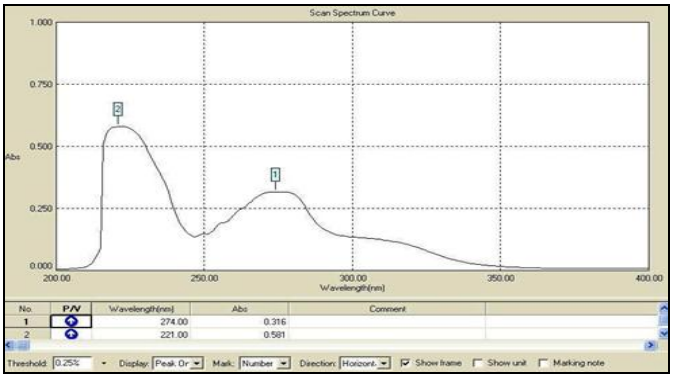

FIG. 4: ABSORBANCE SPECTRA OF *CANNABIS* CYCLOHEXANE EXTRACT

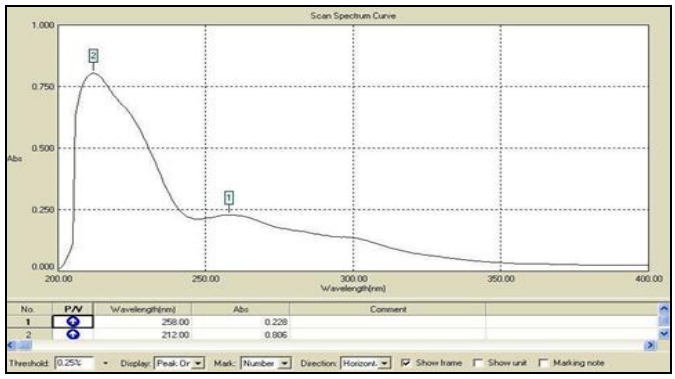

FIG. 5: ABSORBANCE SPECTRA OF *CANNABIS* METHANOL EXTRACT

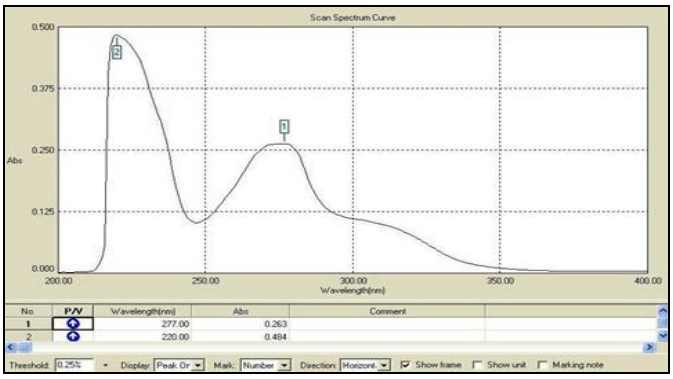

FIG. 6: ABSORBANCE SPECTRA OF *CANNABIS* PETROLEUM ETHER EXTRACT

The quantitative estimation of proteins by Folin's method showed a high concentration of proteins in all the samples as shown in **Table 2**. The concentration of ricin in case of *Ricinus communis* seeds was found to be much higher in all the samples as compared to that found by Al-Mamun *et al.* [9] The standard calibration graph for *Ricinus communis* has been shown in **Fig. 7**. Amount of protein in fresh seeds of *Ricinus* was found to be more as compared to dried seeds. Similarly, the amount of protein in the undiluted extract of *Cannabis* seeds was found to be more than that in the diluted extract. The protein concentration found in *Cannabis* seeds were similar to that found by Tariq and Reyaz [10].

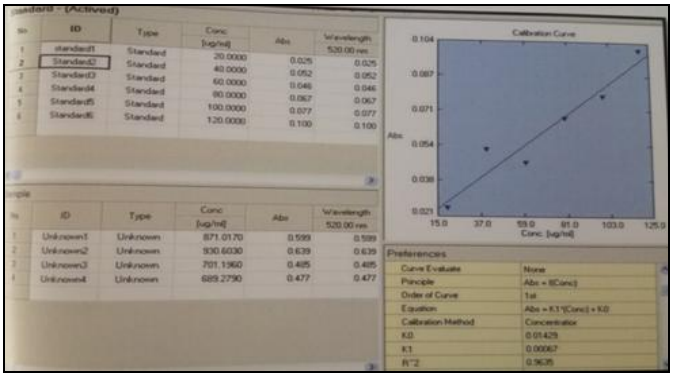

FIG. 7: CALIBRATION CURVE

TABLE 2: CONCENTRATION OF PROTEINS IN DIFFERENT SAMPLES OF *RICINUS* AND *CANNABIS* BY FOLIN'S METHOD

S. no.	Extract	Concentration (µg/mL)
1	*Ricinus* fresh seed sample 1	871.01
2	*Ricinus* fresh seed sample 2	930.60
3	*Ricinus* dried seed sample 1	701.19
4	*Ricinus* dried seed sample 2	689.27
5	*Cannabis* undiluted sample	441.11
6	*Cannabis* diluted sample (1:100)	377.00

Proteins from dry seeds and fresh seeds of *Ricinus* were separated by SDS-PAGE. It was observed that the protein profile of fresh ricin seeds showed faint and comparatively light bands in contrast to dried seed samples in which relatively darker bands were observed. In both the protein profiles (fresh and dried seeds) bands ranging from 45kDa to approximate 15kDa were observed which were in accordance with the results as obtained by Al-Mamun *et al.* [9] In the protein profile of fresh seeds, bands of molecular weight of 43kDa, 44kDa, 25kDa, 24kDa, 23kDa, 17kDa, 16kDa, 15kDa, were observed. A similar observation was made in the protein profile of dry ricin seed sample, wherein bands of 43kDa, 44kDa, 25kDa, 24kDa, 23kDa, 17kDa, 16kDa, 15kDa, were observed. However, bands of 46kDa and 19kDa were observed only in the dry seed sample which was not present in the fresh seed sample. Some of the protein extracted from Cannabis resolved from 180kDa to 11kDa. The protein was observed to be resolved at 180-100kDa, 45kDa, 40kDa, a dark band at 24kDa, 14kDa, 12kDa similar to that as found by *Sunday* Abiodun Malomo [11]. The results are given in **Table 3** and **Fig. 8** and **9**.

TABLE 3: MOLECULAR WEIGHT (kDa) OF PROTEINS SEPARATED FROM *RICINUS* AND *CANNABIS* BY SDS-PAGE

S. no.	Sample	Molecular Weight (kDa)
1	*Ricinus* (fresh seed sample)	43kDa, 44kDa, 25kDa, 24kDa, 23kDa, 17kDa, 16kDa, 15kDa
2	*Ricinus* (dried seed sample)	43kDa, 44kDa, 25kDa, 24kDa, 23kDa, 17kDa, 16kDa, 15kDa
3	*Cannabis*	Some bands resolved between 180-100kDa and rest at 45kDa, 40kDa, 24kDa, 14kDa and 12kDa

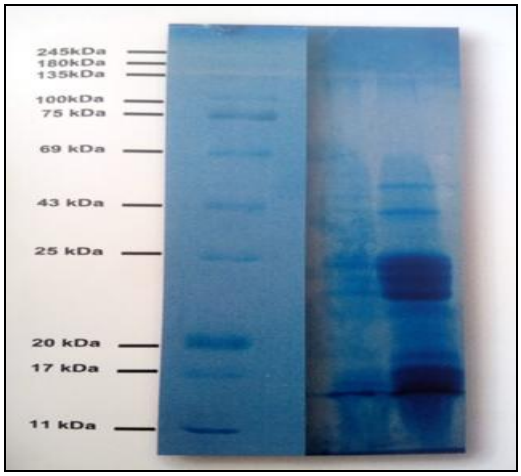

FIG. 8: SDS PAGE OF *RICINUS COMMUNIS*

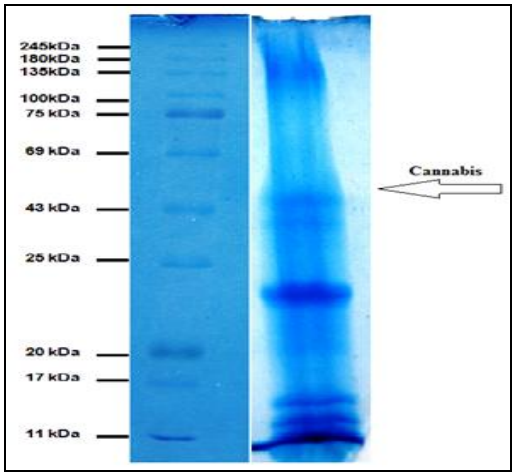

FIG. 9: SDS PAGE OF *CANNABIS SATIVA* L.

CONCLUSION: Plants contain proteins in large amount. Protein content and size vary among every plant. As some people use different parts of *Cannabis* to prepare its decoction and tea and use it as a herbal remedy to treat many of the ailments, [12] estimation of protein becomes highly important in this case. In the case of *Ricinus*, the Castor oil is commonly used to prepare hair oils. There may be chances of some amount of ricin protein present in the oil extract from the seeds which is a toxic substance, and therefore it necessitates the need for its estimation through proper methods. Thus, we see that protein estimation, isolation and characterization are important aspects for all the botanical samples. UV spectrophotometric method can be used for both qualitative as well as quantitative analysis of proteins. Apart from UV, SDS-PAGE can be considered as one of the best method for the separation of protein based on its size. From our study, we were able to separate the protein from seeds of *Ricinus communis* and *Cannabis sativa* ranging in size from 45kDa to approximately 15kDa and 45kDa to 10kDa respectively. Thus, both the methods proved simple, efficient and convenient to perform that can be performed in the regular laboratory analysis in a variety of laboratories and research and development fields.

ACKNOWLEDGEMENT: The authors are thankful to the Institute of Forensic Sciences for providing the necessary infrastructures and facility for completing the research work.

FINANCIAL SUPPORT AND SPONSORSHIP: Nil

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

REFERENCES:

1. Raharjo TJ, Widjaja I, Roytrakul S and Verpoorte R: Comparative proteomics of *Cannabis sativa* plant tissues. Journal of Biomolecular Techniques 2004; 15(2): 97-106.
2. Cronquist A: The Taxonomic significance of the structure of plant proteins: a classical taxonomist's view. Brittonia 1976; 28(1): 1-27.
3. Coyle MH, Lee CL, Lin WY, Lee HC and Palmbach TM: Forensic botany: using plant evidence to aid in forensic death investigation. Croat Med J 2005; 46(4): 606-12.
4. ST/NAR/40; Recommended Methods for the Identification and Analysis of *Cannabis* and *Cannabis* Products.
5. Small E: The case of the curious Cannabis. Econ. Bot. 1975; 29: 254.
6. https://en.wikipedia.org/wiki/Ricin.
7. Tyagi K, Sharma S, Rashmi R and Kumar S: Study of phytochemical constituents of *Ricinus communis* Linn. under the influence of industrial effluent. JPR 2013; 6: 870-3.
8. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227(5259): 680-5.
9. Al-Mamun: Characterization and evaluation of antibacterial and antiproliferative activities of crude protein extract isolated from the seed of *Ricinus communis* in Bangladesh BMC Complementary and Alternative Medicine 2016; 16: 211.
10. Tariq AL and Reyaz AL: Isolation of Cannabinoids from the plant *Cannabis sativa* and its potential anticancer activity. Int J Drug Dev. & Res 2012; 4(1): 241-6.
11. Malomo SA: Structure-function properties of hemp seed proteins and protein-derived acetylcholinesterase-inhibitory peptides. Ph.D. Thesis, Faculty of Graduate Studies of The University of Manitoba, Department of Human Nutritional Sciences, University of Manitoba, Winnipeg 2015.
12. Ryz NR, Remillard DJ and Russo EB: Cannabis roots: a traditional therapy with future potential for treating inflammation and pain. *Cannabis* and Cannabinoid Research 2017; 2(1): 210-216.

How to cite this article:
Pandey A, Pendarkar D, Patel AD and Agrawal S: Estimation of proteins in plants of forensic significance (*Ricinus communis* and *Cannabis sativa*) by UV-spectrophotometry and gel electrophoresis method. Int J Pharm Sci & Res 2019; 10(3): 1518-23. doi: 10.13040/IJPSR.0975-8232.10(3).1518-23.