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Evaluation of staining procedures for foldscope based identification of cereal grains and oilseed cakes

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Abstract
Foldscope is a cheap, portable paper microscope with 140 X magnification and has potential to be used as a simple tool for microscopic identification of feed ingredients on the spot under field conditions if appropriate staining procedures are available. In this context, the present study was conducted to evaluate the suitability of iodine and Coomassie brilliant blue staining for foldscope based identification of cereal grains and oilseed cakes, respectively. Starch rich ground maize, sorghum and rice broken were stained with iodine solution. Protein supplements such as soyabean meal, sunflower cake, groundnut cake, rapeseed meal, decorticated cottonseed cake and un-decorticated cottonseed cake were stained with Coomassie brilliant blue solution. The air dried stained samples were examined under foldscope. The colour of endosperm of maize and sorghum was dark blue while the endosperm of rice was in light violet colour under foldscope. Further, the size of the endosperm of rice was large as compared to maize and sorghum. Foldscope images of protein bodies of oilseed cakes stained with Coomassie brilliant blue appeared greenish blue to dark blue colour. Disturbance in cellular structures was noticed in the foldscope images of most of the oilseed cakes. Iodine staining may be useful for foldscope based identification of cereal grains like maize and sorghum in the mixed feed through recognition of blue colour. Further, rice grain may be differentiated from maize and sorghum grain based on the differences in size and colour of the endosperm in foldscope images. However, Coomassie brilliant blue solution may not be useful for foldscope based identification of oilseed cakes as it disturbed the cellular structures and blue colour formation was not specific in the foldscope images of most of the oilseed cakes.

Keywords: Coomassie brilliant blue, foldscope, feed, iodine, protein, starch

1. Introduction
Feed microscopy is one of the methods used for evaluation of quality of feeds. Feed microscopy can be used for identification of feed ingredients, contaminants and adulterants in feeds (Khajarern and Khajarern, 1999) [9]. Surface features are considered in stereomicroscopy while cellular attributes are used in compound microscopy. Microscopy requires less time and less expensive as compared to chemical analysis (Khajarern and Khajarern, 1999) [9]. However, microscopy is not generally considered in quality control of feeds under field conditions as it is not portable. Recently, a portable, cheap paper microscope with magnification power of 140 X known as foldscope has been developed (Cybulski *et al*., 2014) [5]. Foldscope, due to its less cost and portable in nature, can be widely used even by livestock and poultry farmers in the field conditions to assess the quality of feeds. However, foldscope images of various dry powdered feed ingredients were not specific enough to identify their presence in a mixed feed. Staining procedures may be needed for each ingredient to distinguish each other in the mixed feed using foldscope (Alexander, 2018) [1]. Various reagents can be used to histochemically stain the cell walls and cell contents of powdered plant materials (WHO, 1998) [13]. Cereal grains and oilseed cakes are commonly used for feeding of livestock and poultry. Cereal grains are rich in starch while oilseed cakes are rich in protein. Amylose in starch exhibits a deep blue colour in the presence of iodine (Ophardt, 2003) [9]. The Coomassie dye binds to proteins through ionic interactions between sulfonic acid groups and protein amine groups through Van der Waals attractions and produces blue colour (Blakesley and Boezi, 1972) [2]. In this context, the present study was carried out to assess the usefulness of iodine and Coomassie brilliant blue (CBB) staining for foldscope based identification of commonly used cereal grains and oilseed cakes, respectively.

2. Materials and Methods

2.1. Feed ingredients collection and processing

Cereal grains such as ground maize, sorghum and rice broken and oilseed cakes such as soyabean meal, sunflower cake, groundnut cake, rapeseed meal, decorticated cottonseed cake and un-decorticated cottonseed cake were collected from three different sources for the evaluation. According to the standard sampling procedure, 250 g sample was collected for each feed ingredient and 25 g sub-sample was taken and crushed with mortar and pestle. The powdered sample was passed through 0.3 mm screen and stored in plastic containers. The samples were processed in duplicate for each feed ingredient in order to obtain six foldscope images per ingredient.

2.2 Slide preparation for untreated samples

A minute quantity of powdered cereal grain or oilseed cake was spread uniformly on a glass slide and covered with transparent cello tape.

2.3. Preparation of staining solutions and slide preparation

2.3.1. Iodine solution preparation

Iodine solution was prepared according to the method of Demarco (2017) [7] with modifications. Based on the experiments conducted to decide the optimum concentration of iodine and potassium iodide, 0.4 g iodine and 0.8 g potassium iodide were taken in a beaker and dissolved using small volume of distilled water. Then, the contents were transferred to 100 ml volumetric flask and the volume made up to the mark using distilled water. The iodine solution was stored in an amber colour bottle in dark place at room temperature.

2.3.2 Iodine staining procedure

A minute quantity of powdered cereal grain (maize, sorghum or rice broken) was taken on a glass slide. A drop of absolute glycerol and two drops of iodine solution were added to the sample with the help of Pasteur pipette and the sample was kept undisturbed for 10 minutes. The excess stain on the slide was removed using tissue paper and the air dried stained sample was uniformly spread and covered with the transparent cello tape.

2.3.3. Coomassie brilliant blue solution preparation

Soyabean meal, sunflower cake, groundnut cake, rapeseed meal, decorticated cottonseed cake and un-decorticated cottonseed cake which are rich in proteins were stained with CBB as described by Cawood *et al*. (1978) [3] and Dong *et al*. (2011) [6] with minor modifications. Fifty milligrams of CBB G-250 was dissolved in 100 ml of distilled water. The resultant CBB solution was filtered through Whatman No.1 filter paper and stored at 4 °C in a refrigerator. This methodology was standardized through conducting several preliminary experiments.

2.3.4. Coomassie brilliant blue staining procedure

A minute quantity of sample (Oilseed cakes) kept on a glass slide was treated with two drops of hot (boiling temperature) CBB solution and kept undisturbed for three minutes .However, the treatment period for rapeseed meal was 10 minutes. The excess stain was removed by adding hot distilled water to the sample and removing the stained distilled water with tissue paper. This washing procedure with hot water was repeated seven times to remove the excess stain. The CBB treated sample on the slide was air dried and covered with the transparent cello tape. The method of application of staining also standardized through several preliminary studies.

2.4. Foldscope examination and image capturing

The foldscopes used for the experiments were supplied by Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi under a Foldscope Project. The processed slide was inserted into the foldscope and viewed using chargeable LED light as light source. The foldscope was attached to smart phone camera and light source using magnetic couplers and the foldscope images were captured with smart phone camera through adjusting the zoom function (Alexander, 2018) [1]. Foldscope images of powdered feed ingredients (Cereal grains and oilseed cakes), iodine treated cereal grains and CBB treated oilseed cakes were recorded using smartphone camera as described earlier.

3. Results

3.1. Foldscope images of powdered cereal grains

The foldscope of images of powdered maize grain, sorghum grain and rice broken are shown in the figures 1A, 1B and 1C, respectively. Endosperm and starch granules of maize were black under foldscope. Starch granules were oval and polygonal. They were distributed mostly in clumps. However, the images were not clear (Figure 1A). The endosperm and starch granules of sorghum also had similar foldscope image characteristics of maize. The starch granules in foldscope images appeared relatively darker in sorghum (Figure 1B). The foldscope images of rice broken showed presence of starch granules and endosperm. The starch granules were not clearly visible and were mostly attached to endosperm (Figure 1C).

3.2. Foldscope images of powdered oilseed cakes

The foldscope images of powdered oilseed cakes (Figures 2A to F) failed to show unique features for the identification of different oilseed cakes. The foldscope images of soyabean meal revealed presence of cotyledons in various shapes with colour ranging from grey to black. However, proteins bodies were not clearly visible (Figure 2A). Groundnut cake cotyledons (Figure 2B) appeared as light yellow to colourless with no specific distinctive features. Protein bodies or starch granules could not be recognized in the foldscope images. Un-decorticated cotton seed cake cotyledons (Figure 2C) appeared dark orange to light yellow colour with smooth surface. Protein bodies were difficult to recognize in the foldscope images of un-decorticated cottonseed cake. However, the fibres were flattened and twisted. The cotyledons of de-corticated cotton seed cake (Figure 2D) appeared structurally similar as un-decorticated cotton seed cake except that they were devoid of fibres and yellow colour. The colour of cotyledons of sunflower cake (Figure 2E) in foldscope images ranged from colourless to grey to black. Protein bodies or other internal structures were not clearly visible. The cotyledons of rapeseed meal (Figure 2F) in foldscope images appeared in different shapes with colour ranging from grey to light yellow while the seed coat had unique reticulated surface with orange colour.

3.3. Foldscope images of powdered cereal grains stained with iodine solution

The starch granules of maize, sorghum and rice in foldscope

images were dark blue in colour due to iodine staining (Fig 3A to 3C). The endosperm of maize and sorghum also appeared dark blue while the endosperm of rice was light violet in colour. Further, the size of the endosperm of maize and sorghum was small as compared to rice. However, the number of starch granules was less in rice as compared to maize and sorghum whereas the number of endosperm fragments was less in maize and sorghum than rice.

3.4. Foldscope images of powdered oilseed cakes stained with Coomassie brilliant blue solution

The protein bodies of oilseed cakes in foldscope images were greenish blue to dark blue colour due to staining with CBB (Fig 4A to F). Foldscope images of soyabean meal (Figure 4A) stained with CBB showed cotyledons along with protein bodies in dark blue colour. In addition, a different light blue colour structure was also observed in the foldscope images of soyabean meal (Figure 4A). Foldscope images of groundnut cake also showed dark blue colour-stained structures representing cotyledons and protein bodies (Figure 4B). Further, a different light colour structure also noticed in the foldscope images of groundnut cake (Figure 4B). The fibres in un-decorticated cottonseed cake (Figure 4C) were colourless while cotyledons with protein bodies were light blue in colour in the foldscope images. In addition, few small dark coloured structures were also noticed. Foldscope images of decorticated cottonseed cake (Figure 4D) was similar to un-decorticated cottonseed cake except that fibres were absent in decorticated cottonseed cake. The CBB staining with sunflower cake (Figure 4E) revealed presence of structures similar to those observed in foldscope image of decorticated cottonseed cake (Figure 4D). The foldscope images of rapeseed meal (Figure 4F) showed three different structures such as cotyledons and protein bodies stained with dark blue colour, an identified structure with light blue colour and the seed coat in characteristic orange colour.

4. Discussion

4.1. Foldscope images of powdered cereal grains and oilseed cakes

The endosperm and starch granules in the foldscope images of dry powdered maize grain, sorghum grain and rice broken (Figure 1A to 1C) did not show any unique identification features. Therefore, it is not possible to identify these powdered cereal grains using foldscope without any specific staining as observed by Alexander (2018) [1]. When observed under compound microscope after clearing with 8% potassium hydroxide, floury starch granules of maize were large, rounded with distinct central hilum and radiating clefts while horny starch granules were smaller, polygonally shaped with a central hilum (Khajarern and Khajarern, 1999) [9]. Similarly, starch granules of sorghum resembled those of maize in general size, range and shape (Khajarern and Khajarern, 1999) [9] while the starch granules of rice broken were very small with a black hilum (Wall and Blessin, 1969) [12].

As reported by Alexander (2018) [1], specific identification features could not be established based on the foldscope images of powdered soyabean meal (Figure 2A), groundnut cake (Figure 2B), un-decorticated cottonseed cake (Figure 2C), decorticated cottonseed cake (Figure 2D) and sunflower cake (Figure 2E) since the cotyledons, protein bodies and seed coat in the foldscope images were not clear. The twisted and flattened fibre structures in the foldscope images of un-decorticated cottonseed cake may some extent be considered as identification feature as it was absent in the foldscope images of other oilseed cakes. The seed coat of rapeseed meal (Figure 2F) had unique orange coloured reticulated surface which could be considered as identification feature of rapeseed meal.

4.3. Foldscope images of powdered cereal grains stained with iodine solution

Amylose in starch is responsible for the formation of a deep blue colour in the presence of iodine (Ophardt, 2003) [10]. Similar to the observations of present study on powdered maize grain, sorghum grain and rice broken, blue colour staining of starch granules due to iodine staining was observed in many of the earlier studies (Evans *et al.*, 2003; Xiao *et al.*, 2006; Priya and Ravindran, 2015; Cheng and Tsai 2016 and Zhao *et al.*, 2018) [8, 14, 11, 4, 15].

Based on the blue staining of starch granules, it may be possible to identify presence of cereal grains maize and sorghum in the mixed feed. However, based on the size of the starch granules and the intensity of blue colour staining, identity of maize or sorghum grain may be difficult. Based on the differences in size and colour of the endosperm, it may be possible to differentiate rice from maize and sorghum grain. The size of the maize and sorghum endosperm appeared small as compared to rice. Further, the endosperm of rice was stained with light violet colour whereas the endosperm of maize and sorghum-stained dark blue colour due to iodine staining which may considered as identification features of rice. Based on the differences in size and colour of the endosperm in foldscope images, it may be possible to differentiate rice from maize and sorghum grain in mixed cereal grains using iodine staining.

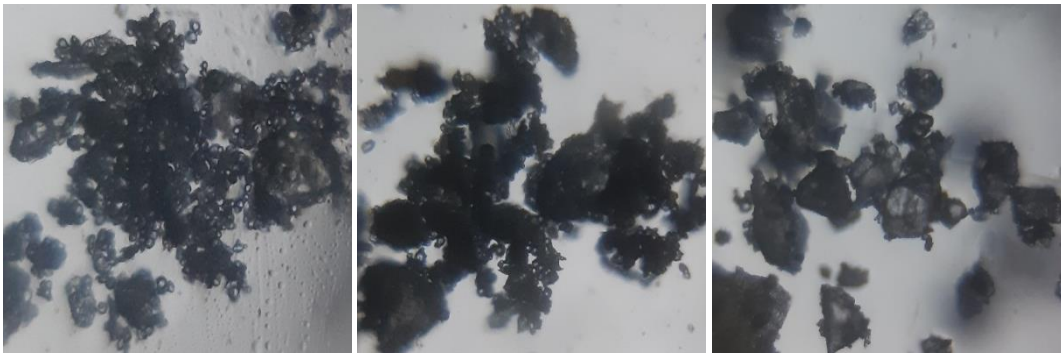
4.4. Foldscope images of powdered oilseed cakes stained with Coomassie brilliant blue solution

It was assumed that as different oilseed cakes contain different levels of protein and the intensity of blue colour on oilseed cakes on staining with CBB in foldscope images may vary with protein content. The variation in intensity of blue colour may be used to identify different oilseed cakes with CBB staining using foldscope. However, in this context, no established CBB staining procedure for oilseed cakes was readily available. Therefore, several experiments were conducted to standardize the CBB staining procedure for oilseed cakes as described in materials and methods.

Disturbance in cellular structures was noticed in the foldscope images of most of the oilseed cakes. Therefore, it was difficult to specify any identification feature based on the cellular structure of for most of the oilseed cakes. Two different characteristic structures with different blue colour intensity in the foldscope images of soyabean meal (Figure 4A) indicated that the intensity of blue colour formed on reaction of CBB with protein depends on the concentration of protein. This was evident from the observation that cotyledons along with protein bodies of soyabean meal were stained with dark blue colour while soyabean hulls (which had different structure) were stained with light blue. Staining of cotyledons and protein bodies in dark blue colour and hulls in light blue colour was also observed in the foldscope images of groundnut cake. However, the hull structure in the foldscope images of soyabean meal was more specific as compared to groundnut cake and hence it may be used as identification feature in soyabean meal.

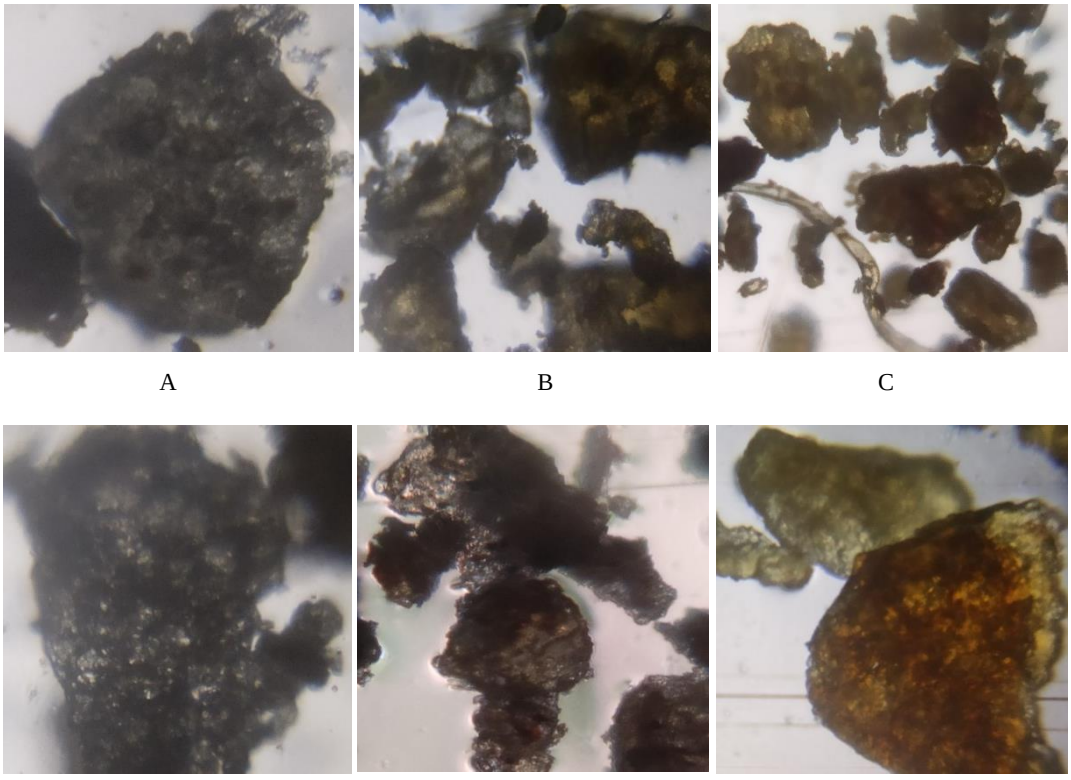
The un-decorticated cottonseed cake had transparent, twisted and flattened fibre structures in the foldscope images (Figure 4C) which can be considered as identification feature since such fibre structure was not observed in foldscope images of other oilseed cakes including de-corticated cottonseed cake. The light blue and light green colour of protein bodies in the foldscope images of un-decorticated cottonseed cake could be attributed to low protein content. The black coloured structure in the un-decorticated cottonseed cake appeared to be cotyledons (Figure 4C).

The CBB staining with sunflower cake (Figure 4E) revealed presence of blue coloured cellular structures similar to those observed in foldscope images of decorticated cottonseed cake (Figure 4D) suggesting that both cakes may have similar protein content. However, specific identification feature for de-corticated cottonseed cake and sunflower cake could not be established from the foldscope images. The characteristic orange coloured structure of seed coat in rapeseed meal (Figure 4F) was not disturbed due to CBB treatment and same can be used as an identification of feature of rapeseed meal. The cotyledons and protein bodies stained with dark blue colour in foldscope images of rapeseed meal which might be due to relatively high protein content in rapeseed meal.



A B C

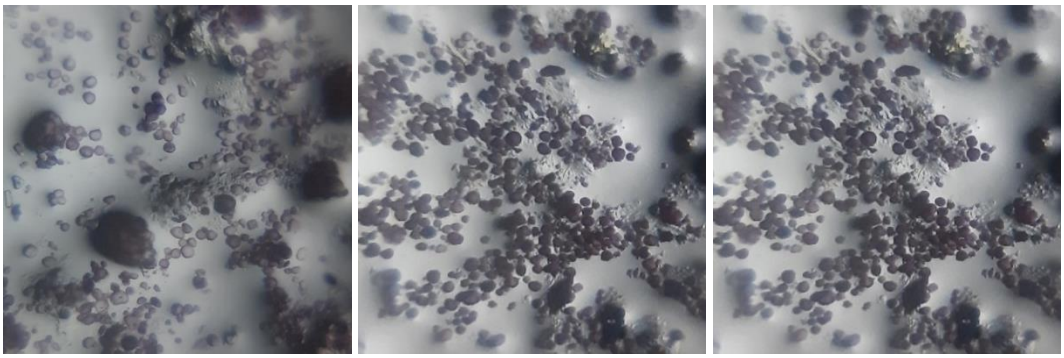
Fig 1: Foldscope images of powdered cereal grains: (A) maize grain; (B) Sorghum grain; (C) Rice broken



A B C

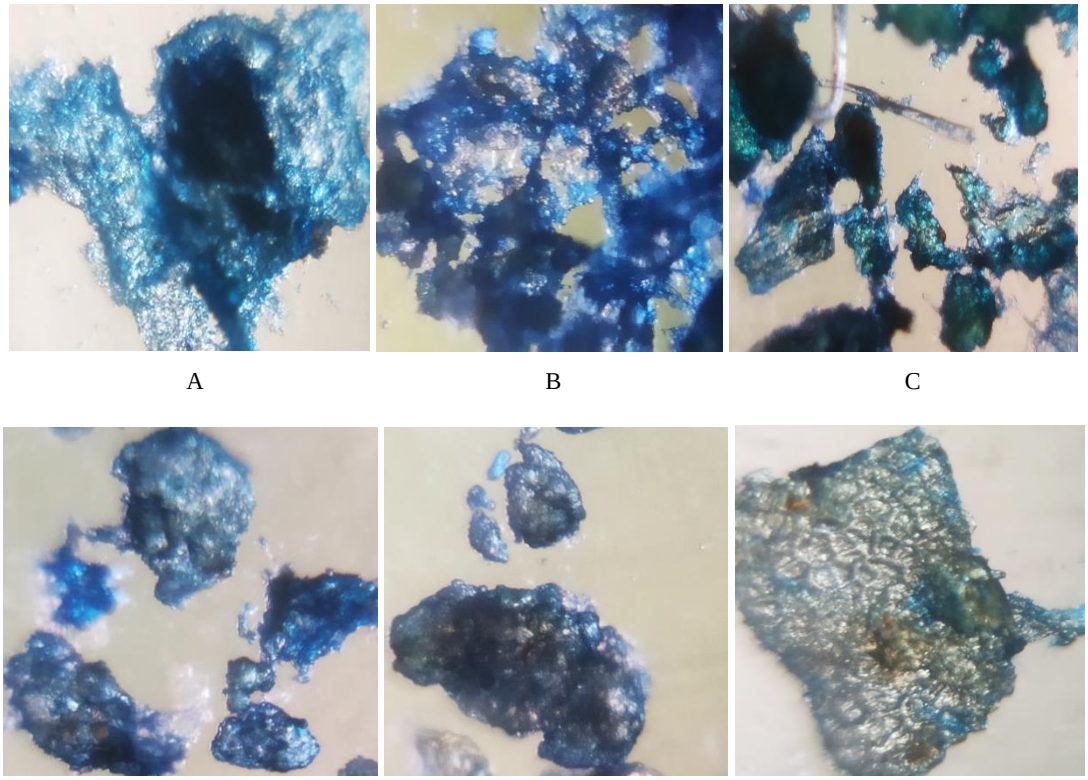
D E F

Fig 2: Foldscope images of powdered oilseed cakes: (A) Soyabean meal; (B) Groundnut cake; (C) Un-decorticated cottonseed cake; (D) Decorticated cottonseed cake; (E) Sunflower cake; (F) Rapeseed meal



A B C

Fig 3: Foldscope images of powdered cereal grains stained with iodine solution:
(A) Maize grain; (B) Sorghum grain; (C) Rice broken



A B C

D E F

Fig 4: Foldscope images of powdered oilseed cakes stained with Coomassie brilliant blue solution: (A) Soyabean meal; (B) Groundnut cake; (C) Un-decorticated cottonseed cake; (D) Decorticated cottonseed cake; (E) Sunflower cake; (F) Rapeseed meal

5. Conclusions

The starch granules of maize, sorghum and rice were stained with dark blue colour due to iodine staining. The endosperm of maize and sorghum were also stained with dark blue colour whereas the endosperm of rice was stained with light violet colour. Further, the size of the endosperm of rice was large as compared to maize and sorghum. Iodine staining may be useful for foldscope based identification of cereal grains like maize and sorghum in the mixed feed through recognition of blue colour. Further, rice grain may be differentiated from maize and sorghum grain based on the differences in size and colour of the endosperm in foldscope images. The Coomassie brilliant blue solution may not be useful to specify identification feature of oilseed cakes as disturbance in the cellular structures was noticed and blue colour formation was not specific in the foldscope images of most of the oilseed cakes treated with Coomassie brilliant blue solution.

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7. References

1. Alexander G. Potential of foldscope for quality control of animal feeds. Proceedings of 11th Biennial Animal Nutrition Association Conference, Patna, India 2018, 31p.
2. Blakesley RW, Boezi JA. A new staining technique for proteins in polyacrylamide gels using Coomassie brilliant blue G250. Analytical Biochemistry. 1977;82(2):580-582.

3. Cawood AH, Potter U, Dickinson HG. An evaluation of Coomassie brilliant blue as a stain for quantitative micro densitometry of protein in section. Journal of Histochemistry and Cytochemistry. 1978;26(8):645-650.
4. Cheng YY, Tsai TH. Analysis of Sheng-Mai-San, a Ginseng-containing multiple components traditional Chinese herbal medicine using liquid chromatography tandem mass spectrometry and physical examination by electron and light microscopies. Molecules. 2016;21(9):1159.
5. Cybulski JS, Clements J, Prakash M. Foldscope: origami-based paper microscope. PloS ONE. 2014;9(6):e98781.
6. Dong WH, Wang TY, Wang F, Zhang JH. Simple, time-saving dye staining of proteins for sodium dodecyl sulfate–polyacrylamide gel electrophoresis using Coomassie Blue. PLoS ONE. 2011;6(8):e22394.
7. Demarco D. Histochemical analysis of plant secretory structures. In: Histochemistry of Single Molecules: Methods and Protocols, Methods in Molecular Biology. Pellicciari, C and Biggiogera, M. (Eds). Springer Science+Business Media LLC. 2017;1560:313-330.
8. Evans A, McNish N, Thompson DB. Polarization colours of lightly iodine-stained maize starches for amylose-extender and related genotypes in the W64A inbred line. Starch-Starke. 2003;55(6):250-257.
9. Khajarern J, Khajarern S. Manual of Feed Microscopy and Quality Control. Third edition. American Soybean Association and United States Grains Council, Thailand, 1999, 6-138.
10. Ophardt CE. Virtual chem book. Elmhurst College. 2003;121-125.
11. Priya E, Ravindhran SR. Pharmacognostical characterization of *Phyla nodiflora* (Verbenaceae): A valuable medicinal plant. Asian Journal of Pharmaceutical and Clinical Research. 2015;8(3):256-261.
12. Wall JS, Blessin CW. Composition and structure of sorghum grains. Cereal Science Today. 1969;14(8):264-268.
13. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva, 1998.
14. Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Analytical Biochemistry. 2006;351(1):146-148.
15. Zhao L, Pan T, Guo D, Wei C. A simple and rapid method for preparing the whole section of starchy seed to investigate the morphology and distribution of starch in different regions of seed. Plant Methods. 2018;14(1):16.