RESEARCH ARTICLE
JIVA 23 (1) April 2025
https://doi.org/10.55296/JIVA/23.1.2025.63-67

JIVA
OFFICIAL JOURNAL OF INDIAN VETERINARY ASSOCIATION, KERALA

Received on 05.04.2025
Accepted on 25.04.2025

RED BEET ROOT (*BETA VULGARIS* L.) PIGMENTS AS AN ECO-FRIENDLY MYCOLOGICAL STAIN FOR VETERINARY DIAGNOSTICS

Athira, K.S[1]., Meghashree Hajong[2], Sreehari, S[2]., Deepa, P. M[3*]., Neethu, P[4]., Athulya E.C[4]., Rathish, R. L[5]. and Janus, A[5]

[1]MVSc scholar, [3]Associate Professor and Head, [4]Research Associate, [5]Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Pookode - 673576
[2]MVSc scholar, Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, College of Veterinary and Animal Sciences, Pookode - 673576

*Corresponding author: deepapm@kvasu.ac.in

ABSTRACT

This study aimed to develop an eco-friendly mycological staining reagent using red pigments extracted from beetroot (Beta vulgaris L.) pomace. Beetroot, rich in water-soluble betalains, was subjected to a blending-based extraction method, which enhanced pigment release, accelerated extraction by increasing surface area, and preserved pigment integrity. The extract was subsequently filtered through Whatman filter paper and stabilized using seventy percent ethanol to enhance colour intensity, improved pigment preservation, and extend stain shelf life. The efficacy of the novel stain was evaluated for the microscopic examination of skin impression smears from dogs suspected of fungal infections. The reagent provided clear visualization of the characteristic morphological structures of *Malassezia pachydermatis*, demonstrating its reliability and safety as a diagnostic stain. This plant-based, non-toxic staining technique offers a sustainable alternative to conventional chemical stains, aligning with the principles of environmental sustainability while ensuring effective fungal identification.

Keywords: Yeast, Malassezia, Dog, Beet root extract

INTRODUCTION

Nowadays, the widespread availability of plant-based products has significantly boosted consumer preference for natural dyes, as they help reduce the health risks associated with synthetic and inorganic dyes. The staining process is a crucial step in microbiological laboratories for studying and identifying microbes, which often involves the use of various inorganic synthetic reagents. Plant-based products can serve as a natural, biodegradable source of chromogenic pigments. Beetroot (*Beta vulgaris* L.), a fleshy, tuberous herb, is the primary source of natural red dye. The key

component responsible for this red pigment is betanin. Betanin is the most abundant of the dyes and was first isolated by Schudel in 1918. The primary crop commercially utilized for betalain production is red beetroot, which contains two main pigments: betanin, a red betacyanin, and vulgaxanthine I, a yellow betaxanthin. Betanin, a red betacyanin, makes up the majority of the pigments, accounting for 75–95% of the total. Vulgaxanthine I is the primary pigment of the yellow betaxanthin group. Another yellow pigment, betalamic acid, is produced through the cleavage of betanin. Previous studies on aqueous extracts of beetroot dye revealed that it contains slightly acidic betalain pigments, making it suitable for use as a mycological stain. This study aimed to substitute the conventional synthetic stain with a natural red dye derived from beetroot pomace. Using beetroot stain, it creates a colour contrast, improving the clarity and detail of *malassezia* organism.

MATERIALS AND METHODOLOGY

A. Collection of plant materials

Fresh beetroot (*Beta vulgaris* L.), weighing 100 grams, was sourced from the local market in Kerala. The samples were thoroughly washed with water to remove any dirt. The beetroot was then peeled, sliced into small pieces, and grated using a blender to increase the surface area for better pigment extraction.

B. Extraction of crude natural dye

The beetroot pulp is placed in a beaker. Seventy percent ethanol is added to the pulp at 1:1 w/v ratio (100 mL of ethanol for 100 g of beetroot). The mixture is then thoroughly stirred to ensure the solvent penetrates evenly. It is left at room temperature for 24 hours, allowing the ethanol to extract the betalain pigments such as betanin and vulgaxanthine (Mini *et al*., 2024).

C. Filtration

The aqueous extract was passed through Whatman No.1 filter paper and then centrifuged at 3000 rpm for 15 minutes to remove all solid residues. The ethanol-based beetroot extract is then collected in a clean beaker. In this experiment, the final solution was adjusted to pH 5 using a citric acid buffer to enhance the dye's stability.

D. Storage

The extracted beetroot stain was stored in a dark glass bottle to protect it from light, which could degrade the pigments. The container was kept in a cool, dark place or refrigerated to extend its shelf life.

E. Staining procedure

Impression smears from dogs suspected of fungal infection were collected from TVCC

pookode. The smears were fixed with methanol for 2–3 minutes and subsequently air-dried. The slides were then flooded with beetroot stain extract and incubated at room temperature for 45 minutes to ensure optimal staining. After staining, the slides were gently rinsed with distilled water to remove excess stain and air-dried. Microscopic examination was performed under 100× magnification (oil immersion) to assess the staining characteristics and morphological details of fungal elements.

RESULTS AND DISCUSSON

Staining is an essential technique in microscopy, enhancing contrast and improving the visualization of microbial structures in biological samples. In microbiology and veterinary diagnostics, stains play a critical role in highlighting cellular morphology for accurate identification. their morphological structures under a microscope. This study evaluated the efficacy of beetroot pomace aqueous extract as a natural fungal stain. Impression smears stained with the crude beetroot extract exhibited satisfactory staining characteristics under 100× magnification (oil immersion), effectively delineating the characteristic peanut- or snowman-shaped budding structures of *Malassezia pachydermatis* (Fig. 1). These findings align with those of Sutradhar and Bhattacharya (2021), further supporting the potential of plant-based staining agents.

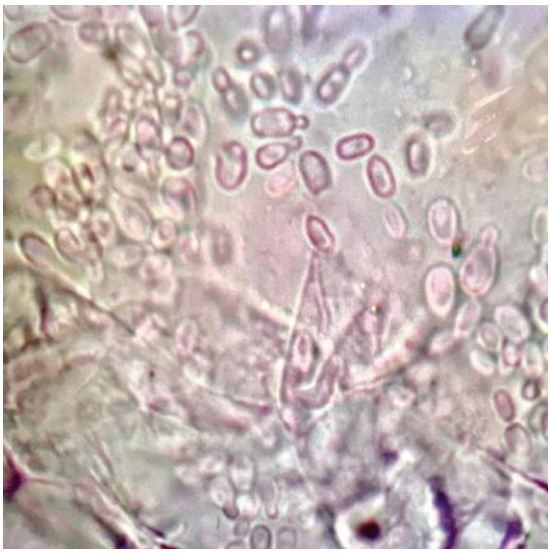

Fig. 1. Photomicrograph of Malassezia pachydermatis stained with prepared beet root extract stain showing budding yeast under 100X magnification

Compared to conventional synthetic stains like Giemsa or Gram's stain, which often contain harsh chemicals or require specialized reagents, beetroot extract provides a natural, non-toxic, and cost-effective alternative with minimal preparation. The staining intensity and clarity were sufficient for microscopic examination, suggesting its applicability in routine fungal diagnostics, particularly in resource-limited settings. However, some limitations, such as variability in staining intensity and the need for further standardization, were noted. Future research should focus on optimizing concentration, pH balance, and staining duration to enhance consistency and contrast.

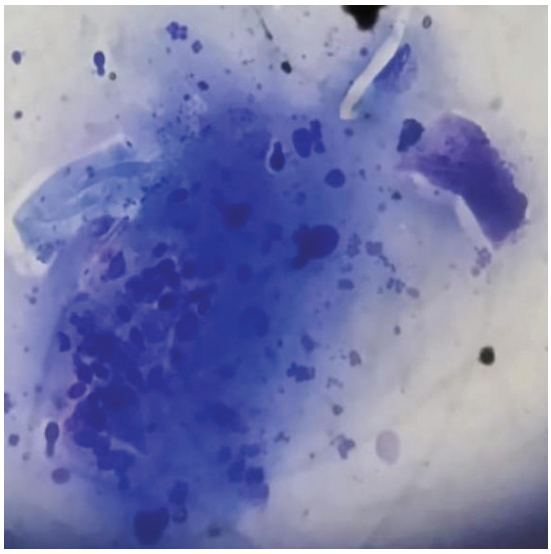

Fig. 2. Photograph of Malassezia pachydermatis stained with Giemsa stain showing budding yeast under 100Xmagnification

These findings highlight the potential of beetroot-derived pigments as an eco-friendly alternative to conventional stains, promoting sustainability in microbiological diagnostics.

CONCLUSION

This study demonstrated that beetroot extract is an effective natural stain for malassezia, providing clear visualization of yeast cells under the microscope. It has the potential to serve as an alternative to conventional synthetic dyes. Additionally, the staining process was simple and did not require complex preparation steps, making it a practical option for routine diagnostic use. The present study concluded that beetroot extract is a safe, eco-friendly, cost-effective, reliable, and biodegradable non-toxic colorant, which holds potential for use as a fungal stain in morphological studies.

REFERENCES

Adeyemo, S. M., Akinloye, A. J. and Adekanmi, G. B. 2017. The Use of Plant Dyes for Microbial Staining and Identification: An Eco-friendly and Non-Toxic Alternative Method. *J. Adv. Biol*, 1-10.

AL-Khateeb, D. S. M. 2019. Extraction Dyes From Two Natural Plants Olive Leaves and Beta vulgaris And The Uses In dyeing Textile.*J. Phys. Conf. Ser*.1294(6), p. 062109.

Cappuccino, J. G. and Sherman, N.1996. *Microbiology - A Laboratory Manual*, The Benjamin/Cummings Publishing Co., Inc., Menlo Park, California.

Das, A., De, D., Ghosh, A., and Goswami, M. M. (2017). Improvement in Cell Imaging by Applying a New Natural Dye from Beet Root Extraction.*1710.08119*.

Mini, C., Nath, S.S. and Lekshmi, S.G., 2024. Standardization of Cold Solvent Extraction Parameters for Natural Red Pigments. *Asian Journal of Dairy & Food Research*, *43*(3).

Pucher, G.W., Curtis, L.C. and Vickery, H.1938. The red pigment of the

root of the beet (*Beta vulgaris*) I. The preparation of betanin. *J. Biol Chem*.**123**: 61–70.

Sutradhar, P. and Bhattacharya, C., 2021. Use of the natural pigments of red beet root pomace (Beta vulgaris L.) to develop a mycological stain: An eco-friendly alternative substitute. *J. Sci. Res*, *65*(03), pp.73-77.

Udonkang, M. I., Inyang, I. J., Ukorebi, A. N., Effiong, F., Akpan, U., & Bassey, I. E. 2018. Spectrophotometry, physiochemical properties, and histological staining potential of aqueous and ethanol extracts of beetroot on various tissues of an albino rat. *Biomedicine hub*, *3*(3), 1-10.