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ALTERNATIVE PROTOCOL FOR NEGATIVE STAINING USING *BETA VULGARIS* EXTRACT

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ABSTRACT

Natural dyes and pigments are of great importance due to their non-toxic and ecofriendly properties. The colour of *Beta vulgaris* roots is an important quality characteristic and thus they are regarded as valuable raw material as natural food colourant, additive and for dye production. This study focuses on the use of beetroot extract at various concentrations as a substitute to negative stains such as nigrosine, which are prepared in harmful chemical solvents. The beetroot extract is a cheaper and greener alternative for use in the laboratory over nigrosine.

INTRODUCTION

Beetroot (*Beta vulgaris*) is being cultivated in many temperate regions for hundreds of years. Beetroot is used as a vegetable and its juice and extracts also as a traditional medicine, food colourant and additive in cosmetics (Kujala *et al*., 2001). The use of beetroot as a source of colour has focused the investigations on betalains (red-violet betacyanins and yellow betaxanthins), which are water-soluble vacuolar chromoalkaloids found in plants of the order *Caryophyllales* as well as in some *Basidiomycota* (Gonçalves *et al*., 2011). According to their chemical structure, these pigments can be subdivided into red–violet betacyanins and yellow betaxanthins (Fig. 1). Betacyanins are derivatives of betanidin, an iminium adduct of betalamic acid and cyclo-DOPA (Gonçalves *et al*., 2011), whereas betaxanthins result from the condensation of α-amino acids or amines with betalamic acid. In nature, betalains occur predominantly in fruits and flowers, including some fluorescent varieties of the latter and around seventy natural derivatives have been described so far (Gonçalves *et al*., 2011). Negative staining procedure is one of the many staining techniques that can be employed for viewing of bacterial cell morphology and size.

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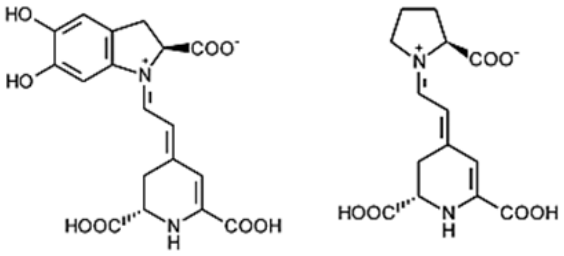
It is a technique by which bacterial cells are not stained, but are made visible against dark background with the use of acidic dyes such as nigrosin. The dye with its negatively charged chromogen does not penetrate the cell due to the repulsion with the negatively charged bacterial cell wall.



Betacyanins **Betaxanthins**

Fig. 1. Chemical structure of betalains in the fully protonated form

It has an advantage over positive staining methods for the study of morphology of cells as they do not receive vigorous physical or chemical treatments (heat fixing or chemical fixing). Nigrosin is a mixture of synthetic black dyes (Solvent black 5) made by heating a mixture of nitrobenzene, aniline and aniline hydrochloride in the presence of a catalyst, Iron

(II) chloride. Sulphonation of nigrosin yields a water-soluble anionic dye known as nigrosin WS (Fig. 2).

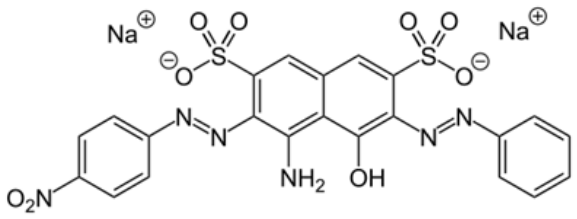
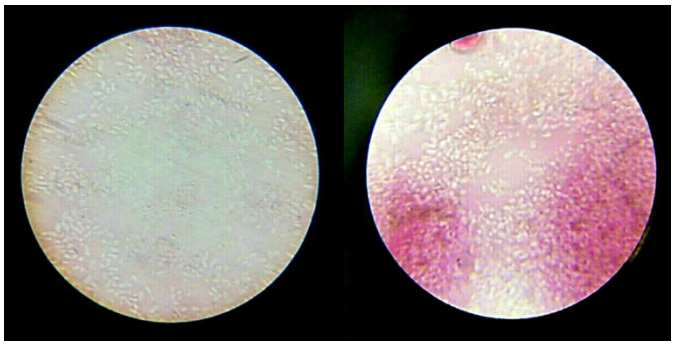


Fig. 2. Chemical structure of nigrosin WS (Acid Black 2), CAS 8005-03-6

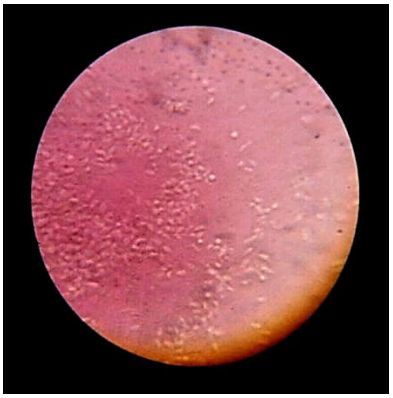
As discussed above, the preparation of nigrosin involves the use of various chemical solvents which have detrimental effects on health and pose as environmental hazards. Betacyanins, which are anionic in aqueous phase can be used as an effective substitute to nigrosin. The water soluble betacyanins in beetroot include betanin, isobetanin, neobetanin and probetanin (López *et al*., 2007; Kujala *et al*., 2001),which can be used for negative staining (Fig. 3). In this study as a substitute for nigrosin, aqueous beetroot extract was used in negative staining procedure.

MATERIALS AND METHODS

Beetroot samples: Fresh beetroots (*Beta vulgaris*, common name red beet), were procured from a local market in Thane, MH, India.

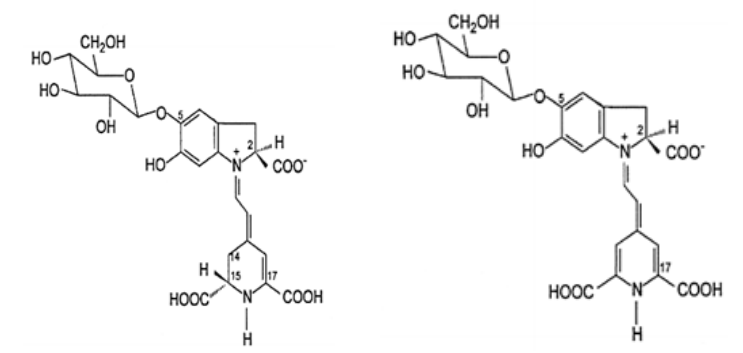


Extract A Extract B

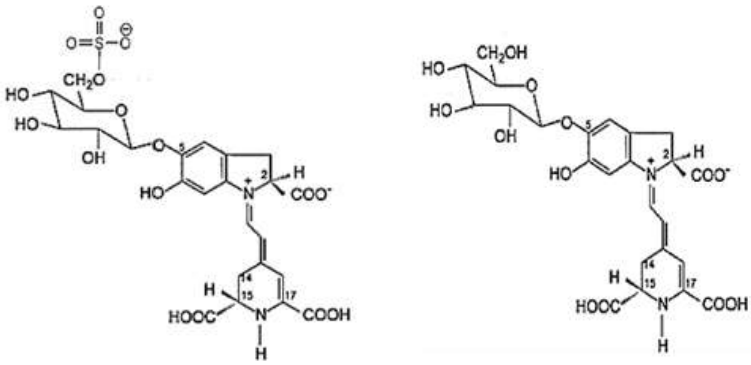


Extract C

Fig. 4. Extract A = crude extract, Extract B = 30-minute concentrate; and Extract C = 1-hour concentration



Betanin **Neobetanin**



Betanin **Neobetanin**

Fig. 3. Common anionic and intensely pigmented betacyanins found in *Beta vulgaris*

Extract preparation : Beetroots (100g) were washed, peeled, sliced and homogenized in a mixer-grinder. The homogenate was prepared in distilled water and then filtered using Whatman filter paper no. 1. 10 ml of this filtrate was poured into clean, dry petri plate and kept in the hot air oven at 50°C for 30 minutes and 1 hour each. The concentrated samples were then used as stains in bright-field negative staining procedure.

Microbial cultures: 24h old culture of *Saccharomyces cerevisiae* was used for the staining procedure.

Staining procedure: Traditional negative staining protocol was used in this study as follows:

- A single drop of each the extract was placed on clean and grease-free glass slide.
- A loopful of the culture suspension was mixed with the sample drop.
- Another clean and grease-free microscope slide was used to smear the extract across the slide evenly.
- The film was allowed to air dry completely and the slides were observed at 45x magnification.

RESULTS

On performing negative staining with the crude and concentrated extracts, colourless oval cells were observed on a pinkish-purple background. The crude extract rendered a very light coloured background which gradually became darker as the extract was concentrated (Fig. 4).

It was noted that the staining capacity of the extract could last only for a few days after extraction, suggesting that freshly prepared extract is optimal for use as a stain (data not shown). This result is in agreement with prior literature on the stability of betacyanins, specifically betanin (Reshmi *et al*., 2012). The extract was found to lose its red-violet colour and turn yellow-brown, indicating a vulnerability to degradation by temperature and light. This yellow-brown extract could not be used for negative staining. In conclusion, our study thus indicates that a natural dye, that does not involve any chemical or harsh physical extraction procedure can be used as a simple and greener alternative to the conventional staining procedure.

REFERENCES

Gonçalves, L.C.P and Bastos, E.L, 2011. "A comparative study of the purification of betanin". *Food Chemistry*.131: 231-238

Kujala, T., Loponen, J. and Pihlaja, K., 2001. "Betalains and Phenolics in Red Beetroot (*Beta vulgaris*) Peel Extracts: Extraction and Characterisation". Z. Naturforsch. 56:343-348

López, J.A.F, Castellar, R., Obón, J.M, and Almela, L., 2007. "Monitoring by Liquid Chromatography Coupled to Mass Spectrometry the Impact of pH and Temperature on thePigment Pattern of Cactus Pear Fruit Extracts". *Journal of Chromatographic Sciences*. 45

Reshmi, S. K., Aravindhan, K. M. and Suganya Devi, P, 2012. "The effect of light, temperature, pH on stability of betacyanin pigments of *Basella alba* fruit". *Asian Journal of Pharmaceutical and Clinical Research*.5:4107-110

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