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The evaluation of *Oryza sativa L* (Black rice) extracts for detection of spermatozoa on the clothing and vaginal swab samples

Korunya Saenguthai, Sirinart Chumean, Chollanot Kaset

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ABSTRACT

Investigation of sexual assault cases from the evidence involving vaginal swab, clothing and others is examined by a forensic scientist. The explanation of trace findings on spermatozoa on clothing is often problematic due to the use of different staining methods. Conventional staining method used either Papanicolaou (PAP) or Dip quick[®] stain as synthetic dyes which are expensive imported material and harmful to human health. Therefore, the present study aims to determine the ability of Oryza sativa L (black rice) extract as a natural dye to detect spermatozoa on the clothing and vaginal swab casework samples for routine forensic examination. Results revealed that black rice extract has a highly effective for detecting spermatozoa on cloth and vaginal swab casework samples. There was no significantly different in the detection of spermatozoa compared with rapid PAP stain and Dip quick[®] stain. Results also showed that the staining of vaginal swab casework with black rice extracted can be used for PCR amplification of centromeric alphoid repeat gene on chromosome Y for 60 days. Moreover, the DNA extracted from stained semen slide generates a full profile of 16 alleles of STR typing. The results indicate that a new natural staining dye which extracted from black rice can be used to detect spermatozoa and identify a person from the trace evidence. The application of natural dyes for routine staining of spermatozoa from forensic specimens will decrease the expense to be spent in purchasing the synthetic dye and reduce their side effects on human and environment. Keywords: Forensic science; Oryza sativa L (black rice); Spermatozoa detection; Staining

method; Natural dyes. 1. Introduction

A sexual assault forensic examination performs both presumptive and confirmatory test on semen evidence from either cloth or vaginal swab of the victim. The presumptive test usually an acid phosphatase (AP) test causes curious results, false-negative results from degradation or false-positive results from vaginal fluid [1]. The positive detection of spermatozoa is therefore a crucial step in determining the investigation strategy for laboratory analysis in sexual assault cases [2, 3]. Positive areas are excised for DNA analysis and further personal identification. Today, spermatozoa detection has been performed under a microscope using histological stains. Since there was no standard dye for spermatozoa staining for using in forensic sciences purpose. Laboratory commonly used Baecchi's, Kernechtrot-Picroindigocarmine (KPIC), Hematoxylin-eosin staining dyes and Christmas tree [2, 4]. However, the tradition staining usually used an expensive imported synthetic materials that can cause an allergic reaction, irritation and burning sensation to eye, skin, nose and throat while some are a carcinogen in nature [5]. Many studies were therefore studied the natural dves for biological samples staining substituted synthetic dves. Previous studies using several plants have been shown that the extracts can be applied in biology and histology staining studies. Curcuma longa (curcumin) extract has been used to stain collagen fibers and red blood cells [6]. Hibiscus sabdariffa (roselle) has the potential for using as a stain for the study of sperm morphology in Sprague-Dawley rats [7].

Rice is a major cereal crop in the developing world and an important staple food source for over half of the world's population [8]. There are many special cultivars of rice containing color pigments such as *Oryza sativa L* (black rice) and *Oryza punctata* (red rice). Reddish

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