# Malaysian Traditional Salads Particles Resembling Microorganisms and Cells in Specimens for Cytology

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**Abstract:** Food contaminants such as vegetable particles in cytology specimens can cause misdiagnosis by mimicking the cells and microorganisms. Misdiagnosis can contribute to false positive or false negative result which then whether will cause delays in diagnosis or an unnecessary treatment thus led to morbidity. The objective of this study is to describe the morphology of traditional salads cells in Malaysia and to identify the mimicking characters of these cells with normal, abnormal and microorganism from cytology specimens. Commonly consumed traditional salads in Malaysia were selected and homogenized using mortar and pestle. This is to resemble chewing affect by breaking down the samples into small particles. Each samples were smeared onto glass slides using back-to-back method. Prepared slides were stained with PAP and MGG stains. The cells were observed and images were recorded. Traditional salads contaminants can resemble *Cocobacillus* sp., *Trichomonas vaginalis*, *Candida* sp. spores, parasites ova, squamous epithelial cells, squamous glandular cells and HSIL. As a conclusion, most of traditional salads cells cytomorphology in this study can mimic human cells and microorganisms in cytology specimens. These finding will provide reference in cytology diagnostic to minimize the rate of misdiagnosis which reassure proper patient treatment.

**Key words:** Cytomorphology, food contaminants, cytology, mimic, traditional salads

## INTRODUCTION

It is necessary to identify contaminants in cytological specimens. Contamination can come from various sources including food. Typically, food contaminants are common in respiratory specimens such as sputum, bronchial washing, bronchial brushing and gastrointestinal tract that can trigger confusion during cytological diagnosis by mimicking microorganisms or cells (Chang *et al.*, 2014; Panthanowitz *et al.*, 2011). Plant cells consist of nuclei, cytoplasm and cell wall. There are few studys identified the basic characteristics of edible plants such as vegetable that can mimic cells and microorganisms in cytology diagnosis (Chang *et al.*, 2014; Saad and Silverman, 2010; Idowu and Powers, 2010; Hughes *et al.*, 2005). As in Malaysia, there is no previous study illustrating scientifically the morphology of local vegetables that can mimic cytology cells. The aim of this study is to provide basic cytomorphology of traditional salads in Malaysia and identify any mimicking characteristics by comparing with various normal cells,

cytopathic changes in cells and microorganism. Traditional salads commonly found in local market was selected which includes petai seeds, Asiatic pennywort leaves, sweet leaf bushes leave, water dropwort leaves, wild cosmos leaves and papaya shoot and flower.

## MATERIALS AND METHODS

The samples were bought from the area of Puncak Alam, Selangor, Malaysia. Traditional salads used were petai seeds, Asiatic pennywort leaves, sweet leaf bush leaves, water dropwort leaves, wild cosmos leaves and papaya shoot and flower. The selected traditional salads were cleaned by using tap water to remove any soil or other unwanted particles. The remaining water from the samples were then dried by air. Only a small part of the samples was taken for processing. To get the resemblance of chewing action, mortar and pestle were used to homogenize the samples. After the process of homogenizing, a smear was made from each of the samples by using back-to-back smear technique. Two

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slides were produced for each samples. All smears were fixed instantly in air-dried for May-Grunwald-Giemsa (MGG) or in 95% ethanol for Papanicolaou (PAP). Staining used in this study were PAP stain and MGG stain. The samples stained using PAP stain and MGG stain were observed and captured using Leica DM750 Microscope equipped with an ICC 50HD camera (Leica Microsystems, Singapore).

## RESULTS AND DISCUSSION

The results showed that vegetable cell could be stained using PAP stain and MGG stain. Plant and animal cell are both eukaryotic cell which shares many similar organelles. Cells of both plant and animal contains nuclei, cytoplasm and nucleic acid. Plant cells differ with animal cells which it has cell wall, the present of chloroplast and vacuole (Bruce *et al.*, 2013). As for morphology, plant cells are significantly different compared to human cell in term of shape, size and structure the cells. Previous study stated that plant cells consists of cell wall which may cause cell to be larger compare to human cells. However, this plant cells such as some vegetable cells said to mimic certain condition during cytology diagnostic and this create concerned (Chang *et al.*, 2014). Based on experimental and observation done in this study, petai cells resembled *Cocobacilus* sp. The mimicking character was shown at Fig. 1. Studies stated that bacterial vaginosis consist of cells enclose with *Cocobacilus* sp. and clue cells with absence of *Lactobacillus* sp. (Karani *et al.*, 2007; Solomon and Nayar, 2004). Petai cells also mimic the appearance of Trichomonas vaginalis as shown in Fig. 1. Petai seeds in PAP stain with 40x magnification Asiatic pennywort cell in Fig. 2 Asiatic pennywort leaves in PAP stain with 40x magnificatio Asiatic pennywort leaves in PAP stain with 40x magnification) and water dropwort cells in Fig. 4 Water dropwort leaves in PAP stain with 40x magnification water dropwort leaves in PAP stain with 40x magnification) resembled endocervical cells with the cytomorphology of honeycombing cells shows by the cell border, hypochromatic eccentric nucleus and it is uniformly in cluster (Cibas and Ducatman, 2009). Sweet leaf bush leaves resembled squamous epithelial cells in Fig. 3 Sweet leaf bush leaves in PAP stain with 40x magnification Sweet leaf bush leaves in PAP stain with 40x magnification). Its Cytoplasm stained greenish on MGG stain, Fig. 3 (Sweet leaf bush leaves in MGG stain with 40x magnification Sweet leaf bush leaves in MGG stain with 40x magnificatio) with rounded, thick-membrane oval structure that may mimic infectious organisms such as
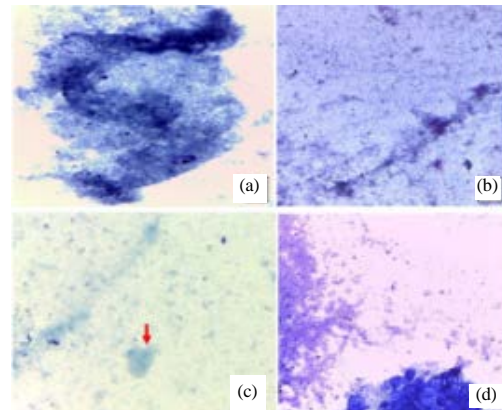


Fig. 1: a) Cells show round cells resembles *Cocobacillus* sp. with conspicuous granular material; b) Particles of granular materials covering the smear background; c) Red arrow shows fibrous materials that may be misinterpreted as Trichomonas vaginalis; d) Basophilic cell with well-defined cell border
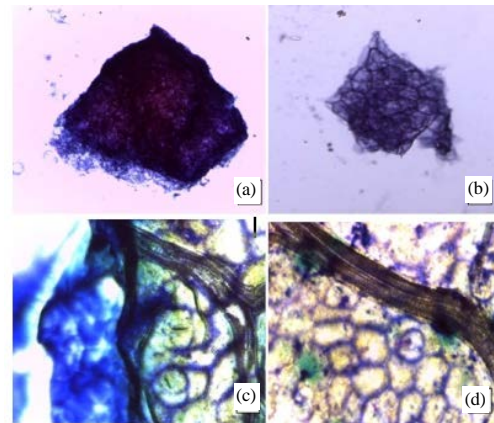


Fig. 2: a) Cells in PAP stain shows a densely basophilic cluster, fine cell walls border; b) polygonal cells with basophilic cytoplasm; c) resembling glandular cells honeycomb-like patterns; d) in MGG stain shows cells with clear-greenish cytoplasm and fine cell wall border with long cylindrical and spiral tubes fibre

*Candida* sp. spores or parasites ova Fig. 3 and 4 (Sweet leaf bush leaves in MGG stain with 40x magnification). As for papaya shoot and flower, pollens of the flower stained with PAP shows hyperchromatic round cells which can be mistaken as either endometrial cells or high-grade squamous epithelial cells (HSIL) Fig. 5 ((a) Wild cosmos leaves in PAP stain with 40x magnification (b) Wild cosmos leaves in PAP stain with 40x magnification
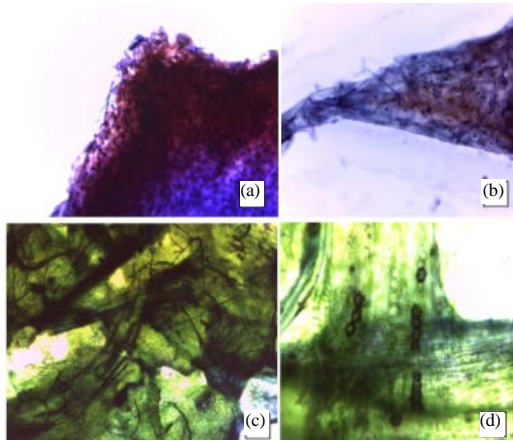
Fig. 3: a, b) Polygonal cells stained purple-blue with centrally-located small hyperchromatic nuclei, resembled squamous cells; c) Cytoplasm stained greenish on MGG stain; d) with rounded, thick-membrane oval structure that may resemble infectious organisms such as *Candida* sp. spores or parasites ova
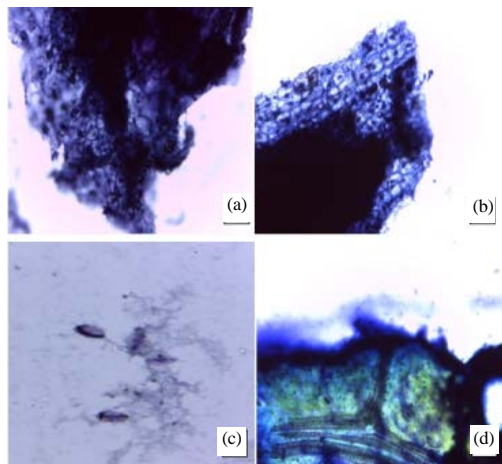


Fig. 4: a, b) Polygonal cells stained purple-blue with centrally located small hyperchromatic nuclei, resembled squamous cells; c) Oval cells pale-basophilic stained; d) Clear-greenish cytoplasm and dense cell wall border with long cylindrical and spiral tubes fibre

(c) Wild cosmos leaves in PAP stain with 40x magnification (d)Wild cosmos leaves in MGG stain with 40x magnification). Fig. 6 ((a) Papaya shoots and flower in PAP stain with 40x magnification. (b) Papaya shoots and flower in PAP stain with 40x magnification). The MGG stain Fig. 6 ((c) Papaya shoots and flower in MGG stain with 40x magnification (d) Papaya shoots and flower in
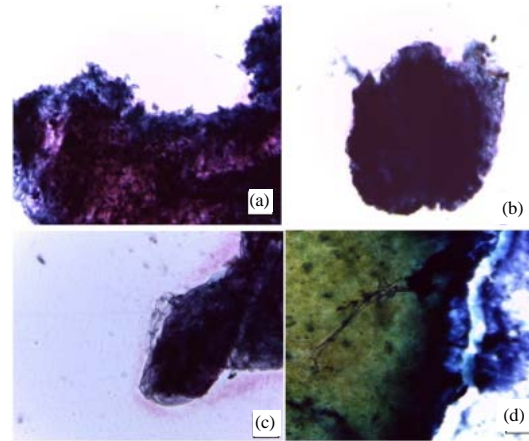
Fig. 5: a-c) Cells show hyperchromatic cells in sheets with defined cell border; d) Greenish cytoplasm and dense cell wall border with long cylindrical and spiral tubes fibr
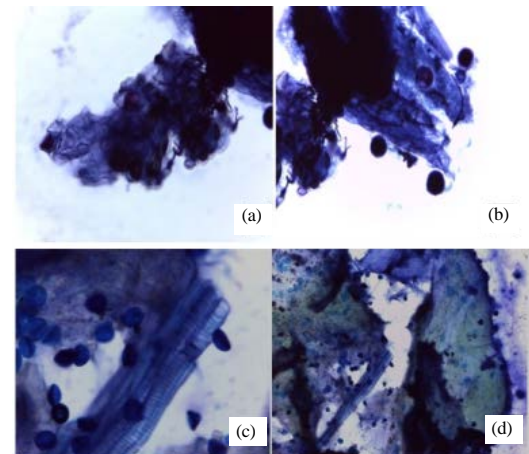


Fig. 6: a, b) Pollens of the flower stained with PAP shows hyperchromatic round cells that can be mistaken as either endometrial cells or High-grade Squamous epithelial cells (HSIL); c and d) The MGG stain shows cells with rounded, oval structure that may resemble infectious organisms such as *Candida* sp. spores or parasite ova

MGG stain with 10x magnification) shows cells with rounded, oval structure that may resemble infectious organisms such as *Candida* sp. spores or parasite ova. Mortar and pestle were used to replicate partial process of digestion and eating effect of local vegetables (Chang *et al*., 2014). However, the possibilities of over crushed of local vegetables might had caused the cells to distort or become fragmented which might have affected the morphology itself.

## CONCLUSION

This study described the basic cytomorphology of traditional salads in Malaysia and highlighted the characteristic comparison and similarity of few of these cells with various microorganisms and human cells. These findings can be used a reference for cytoscreener in reducing the error in cytological diagnostic.

## ACKNOWLEDGEMENT

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