EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 8
306-308
Year: 2019

# STAINING REACTION OF ETHANOLIC EXTRACT OF *CINNAMOMUM TAMALA* IN PROKARYOTES AND EUKARYOTES

**Prianka Dikhar[1], Soumali Sadhukhan[1] and Satadal Das[2]***

[1] Department of Microbiology, St. Xavier's College, Kolkata.
[2]Department of Microbiology, Peerless Hospital & B. K. Roy Research Centre, Kolkata.

***Corresponding Author: Dr. Satadal Das**
Department of Microbiology, Peerless Hospital & B. K. Roy Research Centre, Kolkata.

**Article Received on 07/06/2019** **Article Revised on 27/06/2019** **Article Accepted on 17/07/2019**

## ABSTRACT
*Cinnamomum tamala* leaves are widely used as spices in foods while they also have many other biological and medicinal activities. There is a short list of few natural products which are commonly used for staining of biological materials. Extract of these leaves was not studied as a staining agent. Thus in this experiment we explored staining reactions of the ethanolic extract of the leaves on different biological materials with an expectation to find out whether it can be used as a staining agent for biological materials. Although all prokaryotes studied failed to take a positive staining reaction except *Salmonella* and *Acinetobacter*, it showed good staining reactions with many eukaryotic epithelial tissue, starch granules and fungi. The most interesting finding was a metachromatic and differential staining reaction of human tissue in histological sections, where all epithelial tissue became red in colour while all connective tissue became deep bluish green in colour. Again it failed to stain the tumour tissue indicating a new way to differentiate normal tissue from cancerous tissue. Thus the findings revealed many possible use of this extract as a stain in future for identification of cells and for diagnosis of human diseases. Further studies may reveal many other biological property of this extract as a stain which are hitherto unknown at present.

**KEYWORDS:** *Cinnamomum tamala*, biological stain, histological tissue.

## 1. INTRODUCTION
*Cinnamomum tamala* is an evergreen tree species, belong to the family Lauraceae which is commonly known as bay leaf. It is also known as *Tejpata* in Bengali, *Tejpat* in Hindi, Punjabi and Urdu and *Tamalaka* in Sanskrit.[1] Bay leaf is found and cultivated throughout the country from tropical areas to sub tropical areas of Himalayas and it is also existing in other Asian countries and Australia.[2] Bay leaf or *Tejpata* is widely used since ancient era for different purposes . Due to its aroma, the leaves are kept in clothes and also chewed to disguise bad mouth odour It is well known for its distinct flavor used as spices in Indian food.[9,10] It has also medicinal properties like anti cancer, anti bacterial, carminative, diuretic, anti flatulent effects and useful in treatment of nausea, mouth dryness, bad odour and so on.[3,4,7] The bay leaf contains so many valuable components that makes it important as spices and also as medicine.[8] The main chemical constituent of *C. tamala* leaves are camphene, myrcene, limonene, methyl ether of eugenol, alfapinene, linalool, bornyl acetate, cinnamyl acetate and benzaldehyde.[1] The bark of the tree possesses cinnamaldehyde which is responsible for aroma but other constituent impart the characteristic odour and flavor.[5,6]

Despite of its characteristic essence and aroma, the natural green color of the ethanolic extract of bay leaf may have some role in staining prokaryotic and eukaryotic organisms and tissues. Thus in this study the dark green extract of *C. tamala* was used as a biologic stain, based on its affinity to the substrate present in prokaryotes and eukaryotes cells or tissues.

## 2. MATERIALS AND METHODS
### 2.1 Collection of *Cinnamomum* leaves
Fresh leaves of *Cinnamomum tamala* (bay leaf) were collected from a commercial storage, which are dried and powered into coarse granules with a mixer and taken for extraction.

### 2.2 Preparation of *Cinnamomum* extract
One gram of the bay leave granules was weighed and taken in a previously cleaned glass container with a screw cap. Then 10 ml of 98% ethanol was added into it and vortex it for five 5 minutes. The container was then kept in the fridge at 8-10°C. The supernatant was used after 10 days by then it became dark green in color and this was used in this study as a coloring agent.

**2.3 Tissue or cell selection**

**2.3.1 Human tissue selection**

Histopathologically confirmed paraffin embedded blocks of prostate tissues, stomach tissues with squamous cell carcinoma were sectioned in a microtome and those histological sections were used for the study. Inclusion criteria included those tissues with adequate epithelium and connective tissue.

**2.3.2 Plant tissue selection**

For plant tissue, we selected two species one is *Allium cepa* (onion skin) and another is *Solanum tuberosum (potato section)*. They were collected from local shop.

**2.3.3 The bacterial species selection**

Nine different bacterial strains used in this experiment were isolated from clinical samples. *Escherichia coli, Staphylococcus aureus, klebsiella pneumoniae, Micrococcus luteus, Streptococcus agalactiae, Acinetobacter baumannii, Salmonella typhi, Pseudomonas aeruginosa, Mycobacteium tuberculosis, Bacillus subtilis.* All these bacterial species were cultured and then identified by standard procedures.

**2.3.4 The fungal species selection**

In this study we mainly used *Aspergillus spp and Candida spp* as fungal species. The *Aspergillus* was isolated as common contaminants of the laboratory media and was identified by standard procedure.

Biological agents other than above like buccal epithelium and blood of human beings were also prepared for the staining purpose after taking consents.

**2.4. Staining procedure**

**2.4.1 Staining of histological slides**

For histological slides, paraffin embedded human tissues were first placed on hot plate to allow the paraffin melt. After 15 minutes the slides were taken away from hot plate and dipped into xylene containing coplin jar. Slides were removed from the jar after 10 minutes and dried the excess xylene with blotting paper. Then slides were again washed with ethanol for 2-5 minutes and after that directly stained with the *C.tamala* leaves extract, waited for 10 minutes and washed away gently with tap water. Slides were kept in a humid chamber for 8-10 minutes, dried, and examined under the oil immersion objective in cedar wood oil.

**2.4.2 Staining of bacteria and *Candida***

All bacterial cultures were available as subcultures maintained in the laboratory**.** Smears of each bacteria prepared on glass slides which contain one drop of saline water with the help of inoculating loop. Waited until it dried completely and then heat fixation was done. Then the smear was stained directly with the extract of *C. tamala* for 10 minutes in a humid chamber, followed by examination under the microscope under oil immersion objective in cedar wood oil.

**2.4.3 Staining of *Aspergillus***

Laboratory maintained subcultures were available in Sabouraud's dextrose agar media, the cultures were scraped with a fungal loop and the materials were placed on glass slides and 1-2 drops of extract placed into it. Then the scraped materials were separated from each other with alpines. Then the scraped materials were covered by cover slips and kept in a humid chamber for 8-10 minutes. The slides then examines under low and then high power objectives of the microscope.

**2.4.4 Staining of plant tissue**

The thin section of potato and the onion skin were stained directly with the ethanolic extract of bay leaf and then observed in low and high power objectives of the microscope.

**2.4.5 Staining of buccal epithelium and human blood film**

The smear of buccal mucosa and blood film were prepared on glass slides and kept for air dry for about 3-5 minutes. Then 2-3 drops of ethanolic extract of *C.tamala* placed into it. Buccal smears and blood films were examined under the microscope as described above.

**3. RESULTS**

**3.1 Staining efficiency with human histological slides**

All epithelial cells were stained with excellent red color with prominent clear outer layers while connective cells or tissues itself stained deep blue-green color (Fig.1). But fat cells and tumour cells did not show any color, they remained unstained.

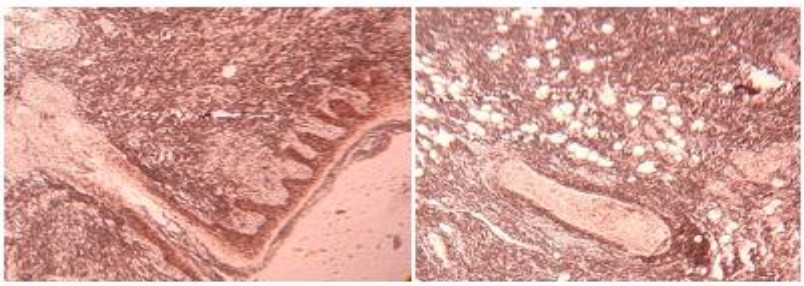

Fig.1: Metachromatic and differential staining of human tissue in histological sections

### 3.2 Performance of *C. tamala* extract on the bacterial cells

The bacterial strains were stained mostly with bluish color. But only two bacterial species *Acinetobacter and salmonella* stained with pink red color. The difference in the colour reaction is not yet clear but it needs further research to know the reason behind it.

### 3.3 Staining reactions with fungal cells

Staining with *Aspergillus* spp showed clear light pink hyphae with clear outer layers, conidiophores stalks were stained with faint pink while the conidiophores itself stained dark red (Fig.2) but smaller spores were unstained. *Candida* spp on the other hand remained unstained.

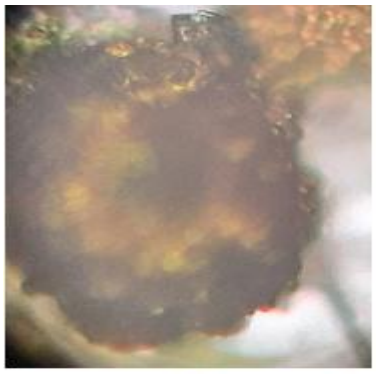

Fig.2: Deeply stained Aspergillus conidiophore

### 3.4 Staining reactions with plant Cells

Staining of potato starch granules showed variegated appearance as dark red and pink mottling with a hazy outlook (Fig.3). Onion skin cells failed to take the stain.

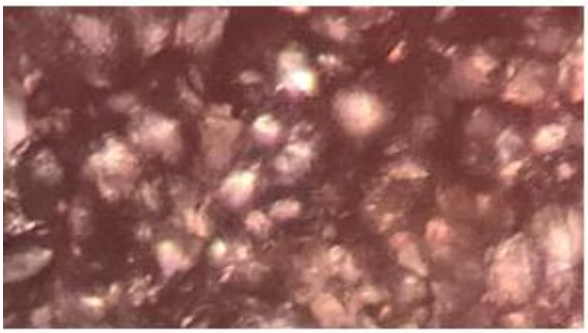

Fig.3: Stained potato starch granules

### 3.5 Staining reactions with buccal mucosa and blood films

RBCs of the blood films were stained with red color but WBCs and platelets remained unstained with *C.tamala* extract. The epithelial cells of buccal mucosa also stained with reddish color.

## 4. DISCUSSION

Staining is the most important and vital part in histology because it is the primary step to identify a species or organisms. However, it involves the use of synthetic stains which pose a possible threat to the ecosystem. Hence, we thought of naturally available stains that could substitute of synthetic or chemical stains. This staining availability of *C.tamala* can be attributed to these chemicals.[8] The extract of the leaves were found effectively stain human histological slides, plant cells, fungal cells, blood film and buccal mucosa epithelium. In case of bacterial strain, though most bacterial cells have taken a faint bluish color, but only *Acinetobacter* and *Salmonella* showed pink color.

Studies on human histological slides have suggested *C.tamala* to be an acidic stain with an increased affinity for epithelial tissue as well as for connective tissue by changing the color in epithelial tissue which was observed as red in color and connective tissue showed dark blue-green color. Such ionic interactions accompanied by non ionic interactions give metachromatic properties of *C.tamala* leaves. This also explains the increased staining affinity of bay leaf toward buccal mucosa cell smear containing epithelial cells.

## REFERENCES


1. D.mal, S.K.Gharde, and R.Chatterjee. Chenical constitute of *C.tamala:* An important tree species. *International journal of Current Microbiology and applied sciences,* 2018; 7(04): 648-651.
2. Showk et al, Chemical composition of essential oil of Cinnamomum tamala Nees and Ebern. *Flavour and fragrance journal,* 2004; 19: 112-114.
3. Megha shah and Mayank Panchal. Ethnopharmacological properties of *Cinnamomum tamala*-a review. *International Journal of Pharmaceutical Rciences Review and research,* 2010; 5(3): Article-025.
4. Kapoor Ld. CRC handbook of Ayurvedic medicinal plants. Florida, CRC press-Boca Raton, 2000; 117.
5. Gaur, R.D.Traditional dye yielding plants of Uttarakhand India. *Natural Products Radiance,* 2008; 27(2): 154-165.
6. Sood RP, Padha CD, Talar IP, Jamwal RK, Chopra MM and Rao PR. Essential oils from the leaves of *Cinnamomum tamala* Nees and Ebern. Growing in Himachal Pradesh, *Indian perfumer*, 1979; 23: 75-78.
7. De Minakhi, Krishna De A and BanerjeeAB. Antimicrobial screening of some Indian species. *Phytother Res.*, 1999; 13.7: 616-618.
8. Vardar Unlu G, Candan, Sokmen A, Domez Eand Tape B.Antimicrobial activity of essential oil from *C.tamala (*Lauraceae). *J Agric food Chem.*, 2003; 51: 63-67.
9. Chakraborty U, Das H.Antidiabetic and antioxidant activities of *Cinnamomum tamala* leaf extracts in STZ-treated diabetic rats. *Global J biotech Biochem,* 2010; 5: 12-18.
10. Gulati BC, Agarwal S, Thappa RK and Dhar KL. *Essential oil of Tejpat from Cinnamomum tamala. Indian perform,* 1977; 21.1: 15-20.