RESEARCH ARTICLE

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# Elemental Composition of Betel Leaves Using a Novel Optical Spectroscopic Technique

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## Abstract

**Objective:** Assess trace elements in betel leaves and slaked lime from different regions of Karnataka, India using Laser Induced Breakdown Spectroscopy (LIBS). **Materials and Method:** Betel leaves from six different regions of Karnataka were obtained and named (for the purpose of the study) BL1, BL2, BL3, BL4, BL5, and BL6 and they were sun-dried. A single tube of slaked lime was obtained from the local 'paan' shop. Each dried leaf and a single blob of slaked lime was subjected to elemental analysis using Laser-induced breakdown spectroscopy. **Results:** A ten-trial experiment was carried out in all six leaves and a blob of the slaked lime. The National Institute of Standards and Technology (NIST) database was used to assess the emission lines. The elements that were predominantly present in all six betel leaves from different regions of Karnataka are calcium, copper, and iron. Slaked lime showed only the presence of calcium. **Conclusion:** It is widely accepted that the consumption of betel quid causes various changes in the oral mucosa including oral potentially malignant disorders (OPMDs) and oral cancer. It is important to analyze each component of betel quid to understand the disease progression. Copper is found to be relatively higher in betel leaves, and it is known that copper-induced fibrogenesis via the lysyl oxidase pathway in oral submucous fibrosis.

**Keywords:** Oral potentially malignant disorders- betel quid- betel leaves- trace elements- LIBS

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## Introduction

Organisms, at the coarse level, are made of compounds of elements in different proportions (Turner et al., 2017). Elements are building blocks of all living beings and they are taken from the environment (Sardans et al., 2012). Thus, the elemental composition of a substance gives us insights into its chemical, physical, and biological properties. According to "World Health Organization (WHO)/Food and Agricultural Organization (FAO)/ International Atomic Energy Agency (IAEA) - An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in the organism." (Mertz, 1998). Trace elements are broadly classified as macro elements (Ca, P, Mg Na, K, Cl, and S) and microelements (Fe, Cu, I, Mn, Zn, Mo, Co, F, Se, and Cr). It is believed that Copper plays a major role in tissue fibrogenesis with the help of Copper dependent enzyme lysyl oxidase which plays an important role in cross-linking collagen and elastin. Copper also binds with the protein product of p53 which causes potentially malignant condition progress into squamous cell carcinoma (Trivedy et al., 2000). The alteration in the level of trace elements in oral cancer was also studied by various authors. The presence of these altered levels of trace elements in serum and saliva in oral submucous fibrosis was correlated to the trace elements present in the betel quid that was consumed by the patients. It is known that dependency to betel quid increase the risk of oral potentially malignant disorders and oral cancer (Sumali R et al., 2021). Among the components in the betel quid, areca nuts supposedly had higher Copper levels (Mohammed et al., 2015). Paan/Betel quid is "a substance, or mixture of substances, placed in the mouth or chewed and remaining in contact with mucosa usually containing at least one of the two basic ingredients, tobacco or areca nut, in raw or any manufactured or processed form" (Patidar et al., 2015). Betel leaf (Piper betle L.,) "is a leaf of the vine of the Piperaceae family. It is an evergreen perennial, dioecious creeper cultivated commercially in South East Asian countries, heart shaped leaf used to wrap arecanut, spices, occasionally tobacco, and mineral lime to form betel quid" (Lalrammawia et al., 2022; Tarafdar et al., 1987).

The need of the study to assess trace elements in each component of betel quid is to understand the source

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of these elements and their role in disease progression. Considering that majority of the individuals suffering from OPMDs and oral cancer share the habits of chewing betel quid, we felt the need to study and evaluate the presence of various trace elements in betel leaves and slaked lime. This experimental study is expected to give us an insight into the role of trace elements in the causation of OPMDs and oral cancer.

## Materials and Methods

*Sample collection*

Betel leaves were obtained from six different regions of Southern Karnataka, India. The region from where the samples were obtained, and their sample name (for the purpose of the study) are listed in Table 1. One tube of slaked lime was obtained from the local "paan shop" in Mangalore, India. The sample was designated as SL1 for the purpose of the study.

*Sample preparation*

The fresh leaves were segregated and sun-dried. It almost took 3 weeks to completely dry the fresh leaves. The dried leaves were stored in an air-tight zip-locked cover and labelled with their sample name. The slaked lime was in a semi-solid consistency. Hence a blob of the slaked lime was solidified using an oven for half an hour.

A single leaf and dried blob of slaked lime was mounted and subjected to a 10-trial experiment and was exposed to a single shot of the laser pulse. Different regions of betel leaf such as mid-rib, veins, and the blade of the leaves were specifically chosen as they showed the most intense emission lines. To choose the most effective range of wavelength of the emission lines, multiple trial runs were carried out in the range between 260-520 nm. The most intense emission lines were present between the range of 310 nm to 337 nm. Hence this range of wavelength was fixed for all the samples of betel leaves. The solidified blob of slaked lime was exposed to a single shot of the laser pulse in different regions. The ten-trial experiment was carried out in the spectral range of 260 nm – 520 nm. The LIBS system uses a high-energy pulsed laser (Nd: YAG) operating at 532 nm with 6 ns pulse width, exposure time of 10 ms and 10 Hz repetition rate to ablate the sample. The recorded spectra from samples are subjected for data pre-processing including baseline correction, normalization, etc. It is followed by analysis based on the known-element database [local and National Institute of Standard and Technology (NIST) database to characterize the samples qualitatively or quantitatively.

## Results

*Betel leaves*

The emission lines of Calcium were perceived to be the most significant in four different wavelengths specifically 315.83 nm, 317.58 nm 317.88 nm, and 335.97 nm, The emission lines of copper were perceived significantly in two different wavelengths specifically 324.39 nm and 327.39nm. The Fe emission line was perceived specifically in a single wavelength of 316.21nm (Figure 1).

*Slaked lime*

The emission lines of Calcium was perceived to be the most significant in ten different wavelengths specifically 315.86 nm, 317.94 nm 370.71 nm, 373.68 nm 393.75 nm

Table 1. Betel Leaves from Different Regions and Their Samples

| Different Regions | Sample Name |
|---|---|
| Kottigehara (latitude – 13.12140N, longitude – 75.52370E) | Betel Leaf 1 (BL 1) |
| Chikkamagaluru (latitude – 13.31610N, longitude – 75.77200E) | Betel Leaf 2 (BL 2) |
| Thirthahalli (latitude – 13.68950N, longitude – 75.24500E) | Betel Leaf 3 (BL 3) |
| Belthangady (latitude – 12.98870N, longitude – 75.5247430E) | Betel Leaf 4 (BL 4) |
| Puttur (latitude – 12.76870N, longitude – 75.20710E) | Betel Leaf 5 (BL 5) |
| Vittal (latitude – 12.76360N, longitude – 75.10160E) | Betel Leaf 6 (BL 6) |

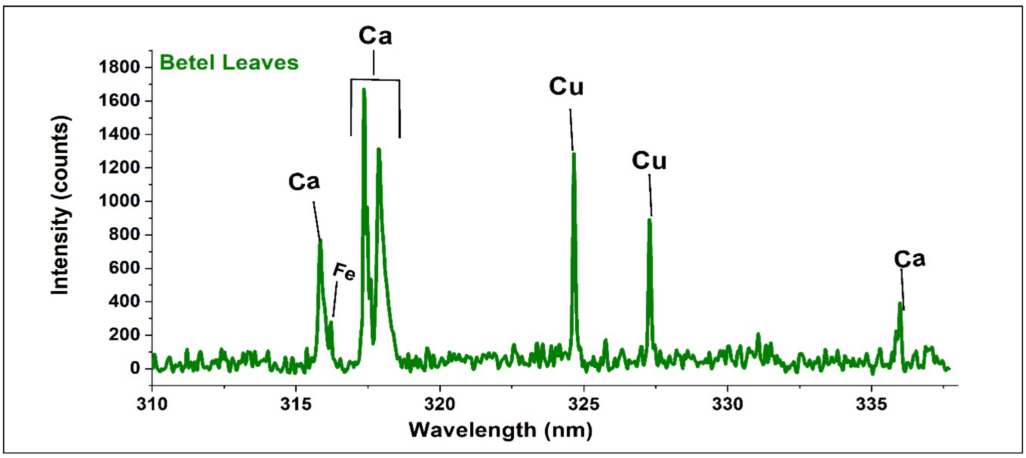


Figure 1. Trace Eelements Recognized Using NIST Database and Characteristics Lines are Marked.

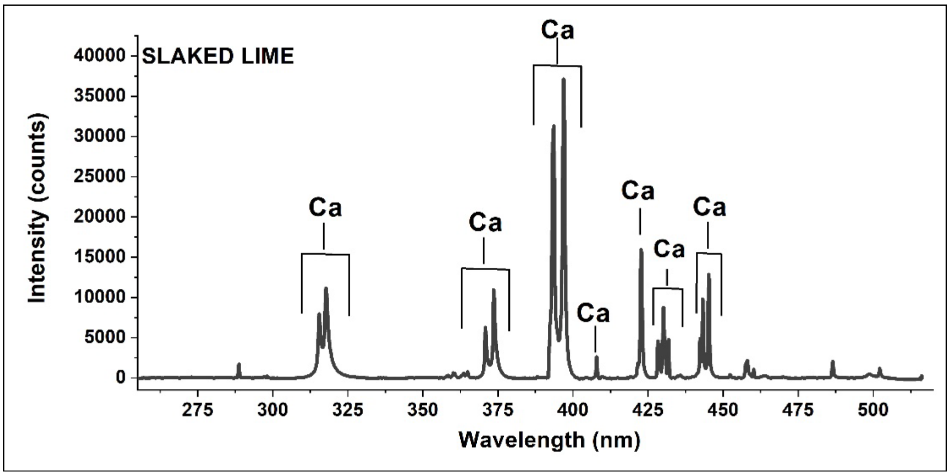


Figure 2. Calcium was the Only Element Present and Denoted as Recognized Using NIST Database.

Table 2. Average Intensity of Trace Elements Present in BL 1

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 379± 46.2 |
| Ca | 317.88 | 1310.77 ± 307.87 |
| Cu | 324.65 | 1120.33± 193.38 |

Table 3. Average Intensity of Trace Elements Present in BL 2

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 405.71 ± 46.87 |
| Ca | 317.88 | 9737.8 ± 2317.43 |
| Cu | 324.65 | 1197.14 ±269.15 |

Table 4. Average Intensity of Trace Elements Present in BL 3

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 212 ±57.43 |
| Ca | 317.88 | |
| Cu | 324.65 | |

Table 5. Average Intensity of Trace Elements Present in BL 4

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 621.85±94.74 |
| Ca | 317.88 | 3370.33±299.28 |
| Cu | 324.65 | 2050.5±428.35 |

Table 6. Average Intensity of Trace Elements Ppresent in BL 5

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 252.33±53.03 |
| Ca | 317.88 | 7930.660±955.82 |
| Cu | 324.65 | 2192±206.65 |

Table 7. Average Intensity of Trace Elements Present in BL 6

| Elements | Most Intense Line (Nm) | Average Intensity (Counts) |
|---|---|---|
| Fe | 316.21 | 376.6 ± 66.31 |
| Ca | 317.88 | 14631.2±2112.06 |
| Cu | 324.65 | 1316.5±206.65 |

396.26 nm, 422.65 nm, 430.53 nm, 431.03 nm, 432.15 nm 442.76 nm, and 445.75 nm and 458.02 nm (Figure 2). The sample had only Calcium and no other additives present.

The average intensity of the most intense emission lines of different elements with their standard deviation of BL1, BL2, BL3, BL4 and BL5 are listed in Table 2 – 7. The intensity of Calcium was greater than Copper and iron was the least in all the samples of betel leaves.

*Comparison of trace elements (Fe, Ca, and Cu) in betel leaves*

The average intensities of the trace elements (Fe, Ca, and Cu) in all six groups of betel leaves were compared using the ANOVA Kruskal Wallis test (Table 8). There was a statistically significant difference between the concentration of trace elements in the six different types of betel leaves (p = 0.001). The median and Interquartile

Table 8. Kruskal Wallis Test to Compare the Level of Ttrace Elements in Betel Leaves

| Elements | Intensity | Median | Interquartile Range | P-value |
|---|---|---|---|---|
| Fe | 374.78± 58.66 | 378 | 217.30 | 0.001 |
| Ca | 8637.98±2292.14 | 8834.23 | 11829.74 | 0.001 |
| Cu | 1685.20± 214.90 | 1683.50 | 1024.74 | 0.001 |

range was higher in Ca compared to Cu and less in Fe compared to Ca and Cu. We can draw the inference that the concentrations of Calcium are higher in betel leaves, then the level of copper. The concentration of iron is least in betel leaves.

## Discussion

We reviewed medical literature in various databases for studies related to the elemental analysis of betel leaves. We believe that trace elemental analysis of betel leaves using Laser Induced Breakdown Spectroscopy is a novel technique and probably first of its kind. There was one study conducted by Shakya et al., (2009) analysed copper levels in betel leaves using atomic absorption spectroscopy (AAS) and there was a higher concentration of Copper about 18.5 ppm and highest compared to other components of the betel quid. Manjesh et al., (2020) compared the level of trace elements in the male and female genotypes of betel vine using AAS. The study concluded that the level of Copper (30.04±2.00 mg/kg) was high in the male genotype when compared with Copper levels in female (24.94 ± 1.32 mg/kg) and other elements present in male and female betel leaves. Our analysis using LIBS in all samples of betel leaves from six different regions of Southern Karnataka was in line with both the studies with regards to the presence of Copper and Iron. Tarafdar et al., (1987) assessed the presence of various trace elements in the component of the betel quid including lime using Proton-induced X-ray emission (PIXE). Betel leaves showed the presence of 15 trace elements namely Ca, Ar, K, Mn, Fe, Cu, Ni, Pb, Br, Pb, Sr, Rb, and Zn. They also stated that no toxic elements were detected in lime. We analysed multiple elements in betel leaves using LIBS and trace elements such as Calcium, copper, and Iron was present with copper levels being relatively higher compared to iron.

In conclusion, there is enough evidence to prove the role of betel quid and commercially packaged forms of tobacco in the causation of oral cancer. However, we believe only some components in the betel quid may be harmful or more damaging than other. Hence its imperative to study individual components of the betel quid and commercially packaged forms of tobacco. Our study on betel leaves showed that calcium and copper levels were relatively high when compared to other elements. Hence, more elaborate studies need to be performed on individual components of commercially packaged forms of tobacco and betel quid.

## Author Contribution Statement

Kripa Adlene Edith A – data curation, methodology, original draft, review, and editing. Ravikiran Ongole - conceptualization, supervision, original draft, review, and editing. Unnikrishnan V K – acquisition, analysis, interpretation of data, review, and editing. Adarsh U K – Methodology, analysis, interpretation of data, review, and editing. Nagaraj N. R – Data collection, review and editing.

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This study was approved by Institutional scientific and ethics committee, Manipal College of Dental Sciences, Mangalore (Protocol Ref No: 20089).

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