Acta Chimica & Pharmaceutica Indica

Acta Chim. Pharm. Indica: 3(4), 2013, 255-260
ISSN 2277-288X

ELEMENTAL ANALYSIS (ICP-OES) AND ANTICARIOGENIC ACTIVITY OF LEAF AND BARK OF *FAHRENHEITIA ZEYLANICA* (THW.) AIRY

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(*Received : 17.09.2013; Accepted : 25.09.2013*)

ABSTRACT

Fahrenheitia zeylanica (Thw.) Airy, belonging to family Euphorbiaceae is distributed in South India. The aim of the presents study was to estimate the content of major and minor elements and to evaluate anticaries activity of the leaf and bark of *F. zeylanica*. The content of 4 major elements and 7 minor elements in the leaf and bark powder was estimated by ICP-OES technique after microwave digestion. Anticaries activity of methanol extract of leaf and bark was determined against 7 clinical isolates of *Streptococcus mutans* by Agar well diffusion assay. The content of calcium and sodium was highest and least respectively among major elements. In case of minor elements, the content of manganese and chromium was highest and least, respectively. The content of all except lithium were high in leaf when compared to bark. Both the extracts were effective against cariogenic bacteria. Bark extract caused high inhibition of bacteria when compared to leaf extract. The plant can be used as a source of important elements required for normal functioning. The plant can be a potential candidate for the development of anticaries agents.

Key words: *Fahrenheitia zeylanica*, Elements, ICP-OES, Dental caries, Streptococcus mutans, Agar well diffusion.

INTRODUCTION

Mineral elements are inorganic substances found in all tissues and fluids of the body. These represent comparatively smaller portion of the diet as compared with major nutrients. Even though mineral elements yield no energy, they are necessary for several biological processes that are essential to life. These mineral elements may be broadly classified as macro (major) or micro (minor) elements based on their daily requirement. The importance of mineral elements is well recognized in human, animal and plant nutrition as their deficiencies in the nutrition can cause a variety of characteristic diseases/disorders. Plant materials form a major portion of diet and their nutritive value is important[1-4].

Diseases of the oral cavity occur in a complex interaction between the host and the bacterial community. Dental caries is most common chronic disease of childhood and can occur in very young children shortly after the eruption of teeth. The disease affects people of all age groups in the world. The disease sets in as the result of a shift in the composition of a biofilm community specific to the human tooth

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surface. Frequent carbohydrate intake can select the acidogenic and acid tolerant species and these species are responsible for caries development. *Streptococcus mutans* appears to be the most common acid producer in caries initiation[5-7]. The herbal remedies have long history for gum and tooth problems in several countries especially in India. In many traditional cultures, the use of herbal chewing sticks for relieving dental problems is common[8,9].

Fahrenheitia zeylanica (Thw.) Airy (syn. *Ostodes zeylanicus* (Thw.) Muell. Arg belonging to family Euphorbiaceae and is distributed in South India. It is distributed in W. Ghats of Wynaad, Anamalais, Atapadi hills of the Malabar and hills of Travancore. It is a lofty evergreen tree[10,11]. The present study was carried out to estimate contents of major and minor elements by ICP-OES and to determine anticaries activity of leaf and bark of *F. zeylanica*.

EXPERIMENTAL

Materials and methods

Collection and identification of plant material

The plant *F. zeylanica* was collected in forests of Hulikal region, Shivamogga District, Karnataka, India during December 2012 and authenticated by Dr. Vinayaka K. S. The voucher specimen (SRNMN/MB/Fz-35) was deposited in the department herbaria. The leaf and bark were shade dried and powdered using a blender.

Estimation of elements

1 g of leaf and bark powder was digested in 10 mL of ultrapure metal free nitric acid in a microwave digester (CEM). After digestion, the content was diluted to 25 mL with distilled water. Estimation of elements was performed using Inductively Coupled Plasma with Optical Emission Spectroscope (ICP-OES, Agilent Technologies 700series, US). The microwave digested sample was aspirated into ICP-OES to estimate macroelements *viz*., Calcium (Ca), Potassium (K), Sodium (Na) and Magnesium (Mg) and microelements *viz*., Manganese (Mn), Iron (Fe), Zinc (Zn), Nickel (Ni), Chromium (Cr), Lithium (Li) and Copper (Cu). The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg/L) in nitric acid[4]. Instrument configuration and experimental conditions are summarized in Table 1.

Extraction

50 g of shade dried and powdered leaf and bark material was extracted with methanol in a Soxhlet extraction assembly. The extract was filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator[12].

Anticaries activity of methanol extract

Potential of leaf and bark extracts to inhibit 7 clinical isolates of *S. mutans* (Sm-01 to Sm-07) recovered from dental caries subjects was determined by Agar well diffusion method. The bacterial isolates were inoculated into sterile Brain heart infusion (BHI) broth (HiMedia, Mumbai) tubes and incubated overnight at 37^{o}C. The broth cultures were aseptically swabbed on sterile BHI agar (HiMedia, Mumbai) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6 mm diameter were punched in the inoculated plates and 100 µL of leaf and bark extracts (25 mg/mL of 25% dimethyl sulfoxide [DMSO]), standard (Streptomycin, 1 mg/mL) and DMSO (25%) were transferred into respectively labeled wells. The plates were incubated at 37^{o}C for 24 hours in upright position and the zone of inhibition formed around the wells was measured[12].

Table 1: ICP-OES Operation conditions

Parameter	Value
Power (kW)	1.2
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Sample flow rate (L/min)	1.5
Replicate read time (s)	3.00
Instrument stabilization delay (s)	15.0
Sample uptake delay (s)	10.0
Pump rate (rpm)	15.0
Rinse time (s)	10.0
Spray chamber	Cyclonic type
Elements, wavelengths (nm)	Ca (422.673), Cu (327.395), Na (589.592), Cr (267.716), Fe (238.204), K (766.491), Mg (279.553), Mn (257.610), Ni (231.604), Zn (213.857), Li (670.783)

RESULTS AND DISCUSSION

In the present study, ICP-OES technique was performed to estimate the major and minor elements present in leaf and bark of *F. zeylanica*. The content of calcium and sodium was highest and least, respectively among major elements and the content of manganese and chromium was highest and least among minor elements in both leaf and bark. The quantities of all elements except lithium were high in leaf when compared to bark (Table 2).

Table 2: Elemental composition of leaf and bark of *F. zeylanica*

Element	Leaf (ppm)	Bark (ppm)
Calcium	16651.05	11246.70
Potassium	12049.55	5406.44
Sodium	154.88	135.72
Magnesium	8605.58	1381.36
Iron	173.90	40.76
Manganese	291.40	99.71
Zinc	16.61	1.23
Copper	6.26	0.82
Lithium	4.43	7.23
Chromium	0.90	0.05
Nickel	19.26	1.39

Anticaries activity of methanol extract of leaf and bark was tested against 7 clinical isolates of *S. mutans*. The zone of inhibition formed around the well was taken as positive for inhibitory activity. The extracts were effective against all isolates of *S. mutans*. Bark extract (zone of inhibition 1.4 to 2.1 cm) was more effective in inhibiting the test bacteria when compared to leaf extract (zone of inhibition 1.2 to 1.6 cm). Inhibition caused by standard antibiotic was higher than that of leaf and bark extracts. There was no inhibition of test bacteria in case of DMSO (Table 3).

Table 3: Anticaries activity of leaf and bark extracts of *F. zeylanica*

Isolates	Zone of inhibition in cm		
	Leaf extract	Bark extract	Streptomycin
Sm-01	1.6	2.1	3.2
Sm-02	1.2	1.5	3.0
Sm-03	1.6	1.6	3.0
Sm-04	1.2	1.6	2.9
Sm-05	1.4	1.7	2.7
Sm-06	1.5	1.9	3.1
Sm-07	1.3	1.4	3.0

In order to meet the requirements for daily activities, all human beings need a number of organic and inorganic nutrients in diet. Carbohydrates, lipids and proteins form the larger part of the diet, consumed in large quantity and are called macronutrients. Vitamins and mineral elements are termed micronutrients as they are consumed in smaller quantities. Both major and minor elements are essential as they are involved a variety of biochemical functions in living organisms. These elements serve as components of enzymes, regulate cellular energy transduction, gas transport, antioxidant defence, membrane receptor functions, second-messenger systems and integration of physiological functions. Some 25 elements have been identified as important for maintenance of human health; therefore, the study of elements in food and plants is of great interest[1,4,13].

In the present study, estimation of mineral elements in microwave digested leaf and bark powder of *F. zeylanica* was carried out by ICP-OES technique. The analytical techniques for estimation of elements are based on atomic spectrometry with single element detection. ICP-OES is advantageous as the technique can estimate many elements at a time. Due of this, ICP-OES is the most widely used analytical technique for elemental determination and many studies have been conducted to validate this method for metal analysis in a large variety of sample types including plant samples[4,13-15]. The content of all except lithium was found to be high in leaf when compared with bark. Among major elements, content of calcium was highest followed by potassium, magnesium and sodium. In case of minor elements, the content of manganese was highest while chromium was found to be in least concentration.

Dental caries is the most common, transmissible, infectious disease of oral cavity. The microflora of dental caries is highly complex and varies among individuals. Mutans group streptococci, in particular *S. mutans* and *S. sobrinus*, and lactobacilli are important bacteria that have been implicated in the initiation and progression of disease. Mutans Streptococci are considered as the principal etiological agents of dental caries[16-18]. Prevention and control of dental caries involves the use of agents such as chlorhexidine, erythromycin, ampicillin and penicillin that have been proven effective. However, excessive use of these chemicals has been reported to cause some undesirable effects such as vomiting, tooth staining or oral

cancer and development of resistant bacterial strains. Due to this, search for alternate strategies for prevention and control of dental caries is of great interest and plants have been one among such alternate sources for the development of anticaries agents[19,20]. A number of studies have been conducted on the efficacy of plants and their metabolites against caries bacteria and the results are promising[8,9,20-24]. In the present study, a marked inhibitory activity of leaf and bark extract of *F. zeylanica* was observed. Anticaries effect was higher in case of bark extract when compared with leaf extract.

CONCLUSION

The plant is found to contain various minerals that are important for several biochemical functions of the body. The plant can be used as a source of important minerals. Marked anticaries activity suggests the possible utilization of the plant to develop anticaries agents.

ACKNOWLEDGEMENT

The authors express thanks to Head, Department of Microbiology, Principal, SRNMN College of Applied Sciences, Shivamogga and NES, Shivamogga for giving support to conduct work. Authors are thankful to Dr. N. Mallikarjun for providing the cariogenic cultures.

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