



Elemental analysis and antimicrobial assay of aqueous leaf extract of *Celtis integrifolia* Lam.

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Abstract

Elemental analysis and *in vitro* antibacterial activity of aqueous leaf extract of *Celtis integrifolia* were investigated. Applying standard methods, the elemental constituents were examined while the antibacterial potentials of the aqueous leaf extract were tested on clinical bacterial isolates using paper disc diffusion method. Elemental analysis showed the presence of Calcium (Ca) 339.5 Mg/L, Sodium (Na) 149.1 Mg/L, Potassium (K) 997.2 Mg/L, Magnesium (Mg) 8.34 Mg/L, Cupper (Cu) 0.140Mg/L, Iron (Fe) 3.17 Mg/L, Zinc (Zn) 0.739 Mg/L and Manganese (Mn) 0.68 Mg/L. Using atomic absorption spectrophotometry, no lethal amount of toxic heavy metals lead and cadmium were found in the leaf extract. The antibacterial activity was tested on *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Shigella dysenteriae*. The results show zero inhibitory activity against the organisms at all the concentrations. It has been concluded that *Celtis integrifolia* contains physiologically important and essential elements like potassium, calcium, sodium and iron; The result showed that aqueous leaf extract of the plant possess no *in vitro* antibacterial activity against the tested microorganisms.

Keywords: Antimicrobial, Aqueous leaf extract, *Celtis integrifolia*, Elemental analysis, Inhibitory activity

Introduction

Celtis integrifolia Lam is commonly known as hackberry or nettle tree. In Nigeria, it is called zuwo, nguzo and wanco in Hausa, Kanuri and Fulfulde languages respectively (Burkill, 1985). It grows to a height of about 26 meters. The leaves are alternate, simple, and 3-15cm long, ovate and have evenly serrated margins (Herbert, 2004). The leaves, root, stem bark and flower are used in the treatment of epilepsy, measles, small pox, chicken pox, gout and control of diarrhoea by locals in some parts of Nigeria (Burkill, 1985). The leaves of *C. Integrifolia* are used for various soup preparations in some parts of northern Nigeria (Matawalli *et al.*, 2014).

Plants are used medicinally and can be a potent source of many drugs (Burkill, 1985). The world health organization (WHO) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care and there are reports on natural substances of plant origin which are biologically active with desirable antimicrobial properties (Mahre *et al.*, 2016). It has been established that medicinal plants are a source of compounds with the potential to act against virulent microbes (Burkill, 1985). The aim of this study therefore is to evaluate the elemental composition

and antibacterial potential of aqueous leaf extract of *Celtis integrifolia*.

Materials and Methods

Collection and preparation of the plant material

Fresh leaves of *Celtis integrifolia* were collected and authenticated at the Department of Biological Science, University of Maiduguri and a Voucher specimen (Species Vet. 208 A) was deposited at the Department of Veterinary Physiology, Pharmacology and Biochemistry herbarium, University of Maiduguri. The leaves were cut into pieces and air dried in the laboratory under room temperature for seven days. The dried samples were grounded into powder using pestle and mortar.

Extraction of plant material

The powdered sample was exhaustively extracted with water using reflux method. Six hundred grams of the powder was put into the flask and processed according to procedure described by Akinyemi *et al.* (2005).

Elemental analysis

Acid digested plant extracts were subjected to Flans Emission spectroscopy (Gallenkanp FGA 330) to determine the presence and concentration of sodium and potassium. Magnesium, cadmium, calcium, iron, lead, zinc, manganese, arsenic, phosphorus and copper presence and concentration were determined using atomic absorption spectrophotometry (AAS) (Philip/pye Unicomp Ltd, England). The elemental concentrations were determined by a standard calibration curve method (Kolthoff & Elving, 1976).

Antimicrobial screening

Source of the bacterial organisms: The stock cultures of the organisms were obtained from the University of Maiduguri teaching hospital. The test organisms were *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Shigella dysenteriae*

Preparation of media:

Nutrient Agar was used for sub culture of *S. dysenteriae*, *B. subtilis* and *S. typhi*, Mannitol salt Agar for *S. aureus*, Eosin methylene blue Agar for *E. coli*, brilliant green agar for *P. Aeruginosa* and Sabourauds dextrose agar for *C. albicans*.

Culture and identification of the organisms: Stock cultures of the organisms were inoculated onto the plates containing the various media. Each organism was put in to a specific media. The plates inoculated

were then incubated immediately for 24 hours at 37°C, the growths were then identified using colonial appearance, Gram staining, examination of the organisms under microscope, and the use of subculture using different media for confirmation of the organisms identified.

Preparation of stock solution of extracts: Stock solution of extracts was prepared by dissolving 0.01g, 0.02g, 0.03g and 0.05g of the extract in 1mL sterile distilled water to give 100mg/mL, 200mg/mL, 300mg/mL and 500mg/mL respectively. An antibiotic of interest, oxytetracycline, at a concentration of 250mg/mL was also used on all the bacterial organisms and the zone of inhibition compared with those of the plant extract.

In vitro antimicrobial sensitivity test: Paper disc diffusion method as described by Kirby *et al* (Kirby *et al.*, 1966) was used to determine the antimicrobial activity of the test extracts. Discs containing different concentrations of dissolved extract were prepared. An overnight culture of each bacterial isolate was spread on the surface of dried nutrient agar plates. The plates were incubated at 37°C for 30 minutes before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 hours after which they were inspected for the zones of inhibitions using a transparent meter rule. The zones of inhibition were measured and recorded in millimeters (mm). The same procedure was carried out with the oxytetracycline (250mg/mL) as control.

Results and Discussion

The result of the elemental analysis of the leaf extract of *C. integrifolia* showed a concentration of potassium ions (997.2 Mg/L), calcium (339.5 Mg/L), sodium (149.1Mg/L), Iron (3.17 Mg/L), Magnesium (8.34 Mg/L), copper (0.140 Mg/L) manganese (0.6 Mg/L), lead (0.042 Mg/L), cadmium (0.001 Mg/L) and arsenic (0.125 Mg/L) (Table 1).

The Potassium content was 997.2 Mg/L. This value is higher than that of *Acalypha wilkesiana* (390 Mg/L) (Madziga *et al.*, 2013). Iron content of the sample was 3.17 Mg/L; which is an appreciable amount. This is lower than *Acalypha wilkesiana* (9.67 Mg/L). As potassium plays a key role in sodium- potassium pump and iron is an integral structural part of haemoglobin and other non-heme proteins, this plant can serve as a supplementary source of these nutritional elements in human diet.

Sodium content of the sample (149.1 Mg/L) was low compared to some common Nigerian vegetables (Madziga *et al.*, 2013). Zinc, iron and manganese are

Table 1: Elemental concentration of *C. integrifolia* leaf extract

Elements	Concentration Mg/L	WHO standard Concentration Mg/L
Calcium (Ca)	339.5	360-800
Copper (Cu)	0.140	1-3
Arsenic (As)	0.125	-
Potassium (K)	997.2	0.1-1.0
Manganese (Mn)	0.68	10-20
Lead (Pb)	0.042	1-2
Cadmium (Cd)	0.001	10-35
Zinc (Zn)	0.739	15-50
Magnesium (Mg)	8.34	-
Iron (Fe)	3.17	0.5-50
Sodium (Na)	149.1	4-5

Table 2: *In vitro* antibacterial activity of aqueous leaf extract of *Celtis integrifolia*

Concentration	Zone of Inhibition					
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. Subtilis</i>	<i>S. typhii</i>
100mg/mL						
200mg/mL						
300mg/mL						
500mg/mL						
Oxytetracycline(250mg/mL)	25mm	28mm	22mm	20mm	22mm	25mm

Key: R = Resistant

dietary trace elements needed for a healthy immune system among other functions. They were all present in appreciable amounts in the plant studied. Calcium content in the plant is reasonably high compared to *Acalypha wilkesiana* (30.89Mg/L) and *Cassia arereh* (105.6 Mg/L) (Olusola *et al.*, 2011). Since the daily requirement of Ca is 1.2g until the age of twenty four, *Celtis integrifolia* should not be taken in large amounts because excess calcium has a negative effect on the heart (Sceriano *et al.*, 1995). Excess calcium may lead to respiratory and cardiac failure (Dukes, 1984). Magnesium content of *C. Integrifolia* was 8.34 Mg/L. this value is higher than *Acalypha wilkesiana* (5.41 Mg/L) and lower than *Cassia arereh* (34.0 Mg/L).

The organisms were found to be resistant to the aqueous leaf extract of *Celtis integrifolia* at all the four different concentrations. Minimum inhibitory concentration therefore could not be calculated. All the organisms were however susceptible to the control (oxytetracycline, 250mg/mL): *S. aureus* 25mm, *S. pyogenes* 28mm, *E. coli* 22mm, *P. aeruginosa* 20mm, *B. Subtilis* 22mm and *S. typhi* 25mm (Table 2). This result showed that aqueous leaf extract of *Celtis integrifolia* possess no *in vitro* antibacterial activity against the tested microorganisms. However, other solvents like methanol, ethanol and ether should be used to prepare the extract. This may produce some

inhibitory effect on the bacterial organisms compared to the aqueous extract used in this study. In conclusion, the result obtained in this work revealed the presence of physiologically important and essential elements like potassium, calcium, sodium and iron. The aqueous leaf extract of the plant did not show any inhibitory effect on the tested bacterial organisms. Earlier works reported the presence of saponins, tannins, reducing sugar, flavonoids and cardiac glycosides in the aqueous leaf extract of *C. Integrifolia*. Some of these chemical substances are shown to have anti-microbial activity against both gram positive and gram negative organisms (Sanni, 2007). However, this result revealed the reason *Celtis integrifolia* is not reported by traditional medicine users to be effective against diseases of bacterial or fungal origin.

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