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Investigation of the Nutritive Value and Mineral Elements of *Combretum molle* Leaves

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Abstract: The study was concern with determining the nutritive value and the concentrations of mineral elements of *Combretum molle* leaves. Leaves were analyzed for mineral elements by using standard analytical methods and AAS. The nutrients (ash, fibre, protein and moisture) content were obtained by different biochemical methods. The leaves were found to be high in Ca (210.01±13.32 ppm) concentration and low in Zn (0.74±0.01 ppm) and Cu (1.89±0.02 ppm) concentrations. The second highest element present was Mn at 66.36±8.21 ppm. Fe was found to be 14.14±2.11 ppm. Carbohydrates, proteins, fibre and moisture were present in significant amount, but low in fat content. The nutritive value of the leaves was found to be 296.49 cal/100 mg.

Key words: *Combretum molle*, mineral elements, nutritive value

INTRODUCTION

Combretum molle is a shrub or small, graceful, deciduous tree 3-13 m high. It belongs to the family Combretaceae which includes 20 genera and about 600 species of plants distributed especially in the tropical and subtropical regions. It is commonly known as basterooibos in Afrikaans and Umbondwe-omhlophe in Zulu. It is a tree of the bush and savannah regions of Africa mostly in the southern foothills of Saudi Arabia, South and Tropical Africa. *C. molle* is used in African traditional medicine for the treatment of fever, abdominal pains, convulsion and worm infections (De Leo *et al*., 2006). It is also reported to be used to treat HIV patients in South Africa. The tree has a long standing reputation for the treatment of liver disease, malaria, tuberculosis, snakebites and general body swellings. Leaves are chewed or pounded, soaked in water and the juice is drunk for chest complaints and as an anthelmintic and sometimes they are used as inhalant in hot steam bath (Bessong *et al*., 2004). Several members of Combretaceae have been used to treat bacterial diseases in southern Africa (Kotzé and Eloff, 2002; Watt and Breyer-Brandwijk, 1962). Antimicrobial activity of six species of Combretum was observed by Alexander *et al*. (1992), whilst on the other hand Breytenbach and Malan (1989) were able to isolate three antimicrobial compounds from *C. zeyheri*. According to Martini and Eloff (1998), *C. erythrophyllum* contains at least 14 antibacterial compounds and some of these were found to have activities higher than chloramphenicol and ampicillin. Eloff (1999) investigated the antibacterial activity of leaf materials from 27 southern African members of the Combretaceae, however, no work has been done on the nutritive value and the concentration of mineral elements of the leaves of *C. molle* and other members of the family.

The nutritive value is an indication of the contribution of food to the nutrient content of the diet. This value depends on the quantity of food which is digested, absorbed and the amount of the essential nutrients (protein, fats, carbohydrates, minerals, fiber and vitamins). Nutritive values of the plants are important as they act as component for human consumption. All human beings require number of complex organic compounds to meet the need for their muscular activities (Benton, 1972). Plant materials form major portion of the diet and therefore, their nutritive value is important (Indrayan *et al*., 2005). Human body comprises chemical compounds such as water, proteins, fatty acids, nucleic acids and carbohydrates and may or may not contain minerals such as calcium, iron, magnesium and zinc (Katzmarzyk and Waist, 2004). Therefore an effort has been made in this study to use appropriate scientific methods to investigate the nutritive value and the concentrations of mineral elements in *C. molle* since deficiency or excess may posses' danger to human (Chapman, 1967).

Minerals are naturally occurring chemical elements the body uses to help perform certain chemical reactions. Minerals form an integral part of functionally important organic compounds such as iron (Fe) in hemoglobin and zinc (Zn) in insulin. They are essential for the normal functioning of muscles, heart, nerves and in the maintenance of body fluid composition among others, as well as for building strong bones. Mineral deficiencies can manifest in different forms of disease

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conditions such as goiter, rickets and other metabolic dysfunction. Minerals are divided into two groups, namely, major minerals and trace minerals. The major minerals include calcium, magnesium, phosphorus, sodium, potassium sulfur and chlorine while the trace minerals include iodine, iron, zinc, selenium, fluoride, chromium, copper, molybdenum and manganese. The body needs larger amounts of major minerals than trace minerals, although trace minerals can be just as important for good health (Chaney, 2006).

MATERIALS AND METHODS

Plant materials: The fully matured leaves of *C. molle* were collected from Limpopo province in Venda village, South Africa from a single tree. The tree was identified by Mr David Phalatsi, senior botanist from North-West University, South Africa and voucher specimen (No. NW 10555) was deposited in the Herbarium of the same department.

Preparation of extracts: Leaves were separated from stems, washed several times with distilled water disinfected with 0.1% $HgCl_2$ solution for 5 min and dried at room temperature. The dried leaves were milled to a fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley and stored at room temperature in closed containers in the dark until used. The powered sample was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. To prepare for mineral analysis, the washed and dried material was taken in precleaned and constantly weighed silica crucible and heated in muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled in a desiccator at room temperature. The carbon free ash was moistened with Conc. H_2SO_4 and heated on hot plate till fumes of sulphuric acid were no longer coming out. The silica crucible with sulphated ash was again heated at 550°C in muffle furnace until weight of the sample was constant. The resultant ash was dissolved in 5 ml of HNO_3/H_2O_2 (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through whatman filter paper and volume was made to the mark with deionized water. The resulting solution was used for elemental analysis by atomic absorption spectrophotometer (Shimatsu, AA 6800) (Aliyu *et al*., 2008). Calibration curves are shown in Fig. 1-5.

Determination of ash content: 10 gram of *C. molle* leaves was weighed in a silica crucible. The crucible was heated first over a low flame until all the material was completely charred then followed by heating in a muffle furnace for about 3-5 h at 600°C. The crucible

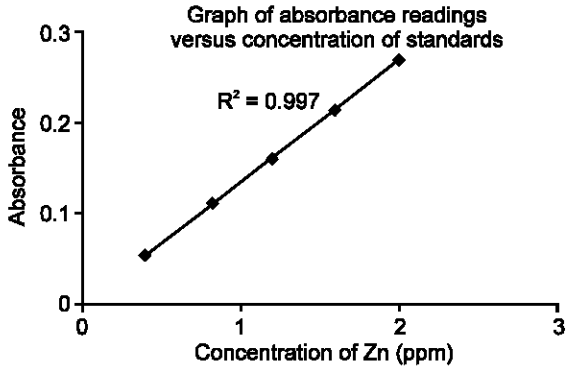

Fig. 1: Calibration graph for Zn standards

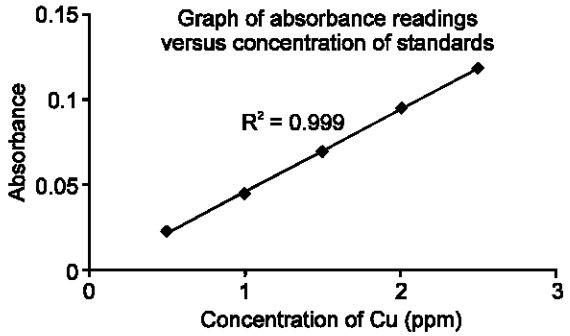

Fig. 2: Calibration graph for Cu standards

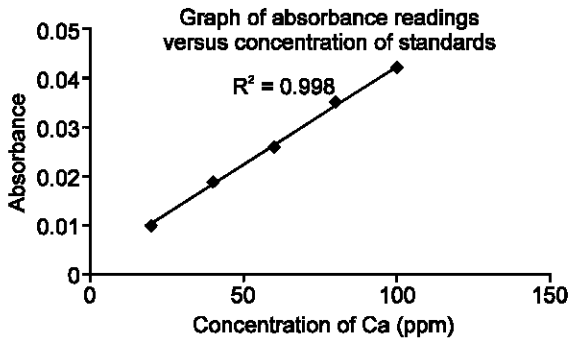

Fig. 3: Calibration graph for Ca standards

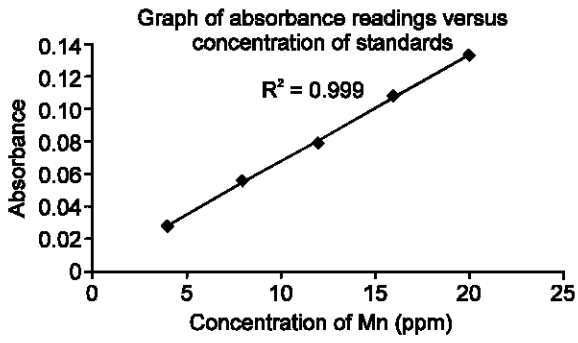

Fig. 4: Calibration graph for Mn standards

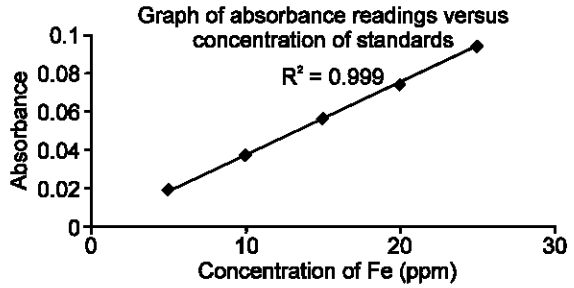

Fig. 5: Calibration graph for Fe standards

was cooled in a desiccator and weighed. The crucible was heated again in the furnace for half an hour to ensure completion of ashing. The crucible was then cooled and weighed repeatedly until the weight becomes constant. Weight of ash gave the ash content that was calculated by the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

Determination of moisture content: Plant material was kept in pre-weighed watch glass and dried at 100-110°C over night in an oven. The sample with watch glass was removed from the oven and placed in a desiccator to cool to room temperature. The sample was re-weighed (this is the dry mass). The loss in weight was regarded as a measure of moisture content.

$$\% \text{ Moisture} = \frac{W1 - W3}{W1 - W0} \times \frac{100}{1}$$

W0 = weight of empty crucible
W1 = Weight of crucible plus sample
W3 = Weight of crucible plus oven dried sample

Determination of crude fat: The fat content was determined gravimetrically after extraction with hexane. 2 gram of moisture-free sample was weighed. 10 ml of hexane was added. The mixture was shaken vigorously and was left to stand for an hour. The residual hexane was filtered using whatman no 40 filter paper and the filtrate evaporated in the pre weighed beaker. The percentage fat content of the sample was calculated by the following formula which gave that the difference in the weights or the original flask and the flask plus extracted fat represent the weight of fat present in the original sample:

$$\% \text{ Fat content} = \frac{(\text{Mass of beaker + fats}) - (\text{Mass of empty beaker})}{\text{Mass of sample added}} \times 100$$

Determination of protein using Kjeldahl method: The sample was first digested in strong sulfuric acid in the presence of 1 g $CuSO_4$ catalyst, which helped in the conversion of the amine nitrogen to ammonium ions. The ammonium ions are then converted into ammonia gas, heated and distilled. The ammonia gas was led into a trapping solution where it dissolved and became ammonium ion once again. Finally the amount of ammonia that was trapped was determined by titration with a standard solution. 2 g of oven-dried sample was taken in a Kjeldahl flask and 30 ml of concentrated H_2SO_4 was added followed by the addition of 10 g K_2SO_4 which raised the boiling point of the digesting acid and the temperature of the reaction. The mixture was heated firstly gently and strongly until frothing ceased. When the solution became clear, it was further heated for another hour. The mixture was allowed to cool, diluted with distilled water and transferred to an 800 ml Kjeldahl flask. 4 pieces of granulated Zn and 100 ml of 40% caustic soda were added to the flask with the splash heads of the distillation apparatus. 25 ml of 0.1 N H_2SO_4 was taken in the receiving flask and distilled. The flask was removed and titrated with 0.1 N caustic soda using methyl red indicator for determination of Kjeldahl nitrogen, which then gave the protein content (Chopra and Kanwar, 1991).

Determination of crude fiber: 2 g of moisture and fat free material was treated with 200 ml of 1.25% H_2SO_4. After filtration and washing, the residue was treated with 1.25% NaOH, filtered, washed with hot distilled water, 1% HNO_3 and again washed with hot distilled water. The residue was ignited and the ash was weighed. Loss in the weight gave the weight of crude fibre.

$$\% \text{ crude fibre} = \frac{(\text{Mass of crucible + ash}) - (\text{Mass of empty crucible})}{\text{Mass of sample taken}} \times 100$$

Percentage of carbohydrate was calculated by the following formula:

$$\% \text{ carbohydrates} = 100 - (\text{Percentage of ash + percentage Moisture + percentage fat + percentage protein})$$

Nutritive value was finally determined by:

$$\text{Nutritive value} = 4 \times \text{percentage of protein} + 9 \times \text{percentage of fat} + 4 \times \text{percentage carbohydrate}$$

Statistical analysis: All determinations were replicated three times and results were reported as mean values ±standard deviation.

RESULTS AND DISCUSSION

Results of the concentrations of the mineral elements are given in Table 1 while the results of various nutrients are summarized in Table 2 and nutritive value in Table 3. The results show that *C. molle* is rich in calcium and manganese. Calcium constitutes a large proportion of the bone, human blood and extracellular fluids (Indrayan

Table 1: Concentrations of mineral elements in ppm

Plant	Zn	Fe	Ca	Mn	Cu
	(ppm)				
Combretum molle leaves	0.74±0.01	14.14±2.11	210.01±13.32	66.36±8.21	1.89±0.02

Table 2: Percentage of ash, moisture, fat, protein, fiber and carbohydrate

Plant	Ash (%)	Moisture content (%)	Crude fat (%)	Crude fibre (%)	Protein (%)	Carbohydrates (%)
CBL	4.03±0.01	22.86±2.21	0.81±0.11	7.80±0.01	7.96±0.03	64.34±3.34

CBL = *Combretum molle* leaves

Table 3: Nutritive value of *combretum molle*

Plant	Nutritive value cal/100 gm
Combretum molle leaves	296.49

et al., 2005). It is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting and also for regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system. Calcium is also essential for disease prevention and control and may, therefore, contribute to some of the traditional medicinal influences of the plant (Aliyu, 2008). On the other hand, manganese is essential for haemoglobin formation (Critchley, 1986) and is an important modulator of cell functions and also play a vital role in the control of diabetes mellitus (Korc, 1988). The results also indicate the presence of iron. Iron in the body makes tendons and ligaments, certain chemicals of the brain are controlled by the presence or absence of Iron and it is also essential for formation of hemoglobin, which carry oxygen around the body (Vaughan and Judd, 2003). Compared to Ca, Mn and Fe, zinc and copper were found in small traces. Copper is a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood (Mills, 1981). The function of zinc in the body is to stabilize the membrane to stimulate immune response (Hambidge, 1978). Its deficiency leads to impaired growth and malnutrition (Prasad, 1981).

The Carbohydrates content of *C. molle* is high compared to the protein and fiber contents. However, it shows that the plant can be used as supplement for carbohydrates, proteins and fiber and therefore might be good to be used as fodder for animals since it has a good nutritive value.

Conclusion: The appreciable concentrations of minerals such as calcium and manganese obtained in the leaves of *C. molle* are interesting. It shows that the plant hold tremendous potential as a source of calcium and manganese. The leaves due to their carbohydrates and proteins contents can be used as fodder for animals. The fiber content in the leaves are able to provide bulk in the diet and this will be able to help to reduce the intake of starchy foods, prevent constipation and reduce the incidence of metabolic diseases like maturity onset diabetes mellitus and hypercholesterolemia.

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