



Determination of Elemental Composition in Some Medicinal Plants Using Instrumental Neutron Activation Analysis

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ABSTRACT

This study investigates the elemental composition of *Artemisia absinthium* and *Boswellia serrata*, two widely used medicinal plants, using Instrumental Neutron Activation Analysis (INAA). INAA revealed a total of 24 elements in both samples, with *A. absinthium* showing significantly higher concentrations of key elements such as aluminum (69,758 ppm), potassium (40,740 ppm), and magnesium (3,703 ppm), while *B. serrata* exhibited elevated levels of calcium (12,350 ppm) and barium (153 ppm). Trace elements like uranium, lanthanum, chromium, and arsenic were also detected, indicating the plants' potential for therapeutic as well as toxicological effects. These findings support the ethno pharmacological uses of both plants and provide a scientific basis for their application in herbal medicine. The comprehensive profiling contributes to quality control, standardization, and further pharmacological evaluation of *A. absinthium* and *B. serrata*.

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INTRODUCTION

The use of plants as medicines is a very ancient story and a traditional medical practice in all the passed civilizations (Samuelsson, 2004). Population rises, inadequate supply of drugs, the prohibitive cost of treatments, side effects of several *allopathic* drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. According to the World Health Organization, 80% of people in developing countries still depend on local medicinal plants to fulfill their primary health needs (World Health Organization, 2002-2005). The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke, 1985).

Due to poverty, ignorance, and unavailability of modern health facilities, most people especially rural dwellers are still forced to the traditional medicines for their common day

ailments, most of these people form the poorest link in the trade of medicinal plants (Khan, 2002). However, *Artemisia* species have been intensively studied in the last decades. *Artemisia* is a large, diverse genus with approximately, 200 and 400 species belonging to the daisy family Asteraceae.

Artemisia is a wind pollinated cosmopolitan genus, mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonizing in arid and semiarid environmental landscape, with only a few representatives in the southern hemisphere. Central Asia is its center of diversification, while the Mediterranean region and North West America are two secondary speciation areas (McArthur and Plummer, 1978; Vales and McArthur, 2001). A few species are also reported in Africa and Europe (Ling, 1994; Shultz, 2006; Tutin *et al.*, 1976). Out of 31 species of *Artemisia* found in India, 21 species are found in Jammu and Kashmir. Many species are economically important as medicines, food, forage, ornamentals or soil stabilizers in disturbed habitats. Some taxa are toxic or

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allergenic while others are invasive weeds which can adversely affect harvests (Pareto, 1985; Tan *et al.*, 1998). Use of plants from *Artemisia* is fairly common in herbal medicine in different parts of the world, and its use goes back beyond recorded history (Hayat 2009c; Ashraf *et al.*, 2010). *Artemisia* species are invariably found as small fragrant shrubs or herbs and most of them yield essential oils.

Artemisia absinthium is an important medicinal plant of family Asteraceae. It has a long-standing traditional use for various indications. The ancient Romans used it for the pain of colitis, an inflammation of the colon. An ethno pharmacy botanical study (1977-2000) confirmed that *A. absinthium* is used in Central Italy for the treatment of lack of appetite (Guarrera, 2005). A survey among Hakims in Pakistan showed that *A. absinthium* is used against liver diseases, hepatitis, blood purification, jaundice, diabetes, skin disease, allergy, scabies, tetanus and as brain tonic (Qureshi *et al.*, 2002). In Russia, Lithuania, Poland and America *Absinthium* herbs are used in folk medicine against dyspeptic complaints (Madaus, 1976). Egyptians used *A. absinthium* as an antiseptic, a stimulant and tonic, and as a remedy for fevers and menstrual pains (Ebers Papyrus). In the middle Ages, the plant was used to exterminate tapeworm infestations while leaving the human host uninjured.

Artemisia Absinthium and *Boswellia* are valued for their diverse medicinal properties. *Artemisia annua*, known for its antimalarial compound artemisinin, is crucial in treating malaria (Meshnick *et al.*, 1996). *Boswellia serrata* is recognized for its anti-inflammatory and analgesic properties due to boswellic acids (Kumar *et al.*, 2010). Understanding the elemental composition of these plants can elucidate their therapeutic potential and enhance their utility in medicinal formulations is of very important.

Neutron Activation analysis was discovered in 1936 when Hevesy and Levi found that samples containing certain rare earth elements became highly radioactive after exposure to a source of neutrons. From these observations they quickly recognized the potential of employing nuclear reactions on samples

followed by measurement of the induced radioactivity to facilitate both qualitative and quantitative identification of the elements presents in the samples.

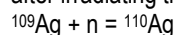
Neutron Activation Analysis (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of major, minor, and trace elements in samples from almost every conceivable field of scientific or technical interest. For many elements and applications, NAA offers sensitivities that are superior to those attainable by other method, on the order of parts per billion or better. In addition, because of its accuracy and reliability.

LITERATURE REVIEW

Theory

In typical NAA, stable nuclides (Z , the target nucleus) sample undergo neutron capture reactions in a flux of (incident) neutrons (David, 2004). The radioactive nuclides ($44Z$, the compound nucleus) produced in this activation process usually decay by emission of a beta particle (β) and gamma ray (γ) with a unique half-life. A high-resolution gamma-ray spectrometer is used to detect these delayed gamma-rays in the presence of the artificially induced radioactivity in the sample for both qualitative and quantitative analysis.

The sequence of events that occur during the most common type of nuclear reaction used for the activation analysis is shown in Fig. 1. The energies of the delayed gamma rays are used to identify component sample elements. The count of gamma rays of specific energies indicates the amount of an element in the sample. For example, when a silver sample is irradiated, a fraction of the ^{109}Ag atoms in the sample will capture a neutron and becomes ^{110}Ag . The radioactive ^{110}Ag atoms have a half-life of 24.6 seconds. Beta decay of ^{110}Ag atoms to ^{110}Cd occurs 4.5% of the time with emission of a 658 keV gamma ray. The amount of silver in the original sample can be determined by measuring the count of 658 keV gamma-rays emitted from the sample in a given time interval after irradiating the sample (David 2004).



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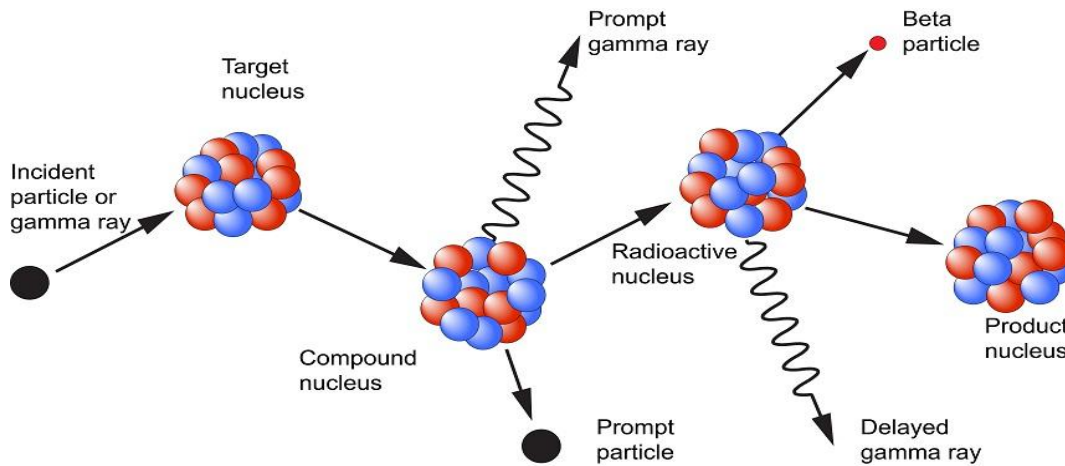
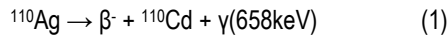


Figure1 Process of neutron capture by a target nucleus followed by the emission of gamma rays (Michael,2007).

The measured count rate R of the gamma rays from the decay of a specific isotope (^{110}Ag) in the irradiated sample can be related to the amount (n) of the original, stable isotope (Ag) in the sample through the equation (2) (Nuclear Science Division and Contemporary Physics Education Project 2000).

$$R = \epsilon I_\gamma A = \epsilon I_\gamma n \psi \sigma (1 - e^{-\lambda t_d}) e^{-\lambda t_d} \quad (2)$$

Where:

R = Measured gamma-ray count rate (cps)
 A = absolute activity of isotope ^{A+1}Z in sample
 ϵ = absolute detector efficiency
 I_γ = absolute gamma-ray abundance
 n = number of atoms of isotope ^{A+1}Z in sample
 ψ = neutron flux (neutrons.cm⁻² sec⁻¹)
 σ = neutron capture cross-section (cm²) for isotope ^{A+1}Z
 λ = radioactive decay constant(s⁻¹) for isotope ^{A+1}Z
 t_d = irradiation time (s)

If the neutron flux ψ , neutron capture cross-section σ , absolute detector efficiency ϵ , and absolute gamma-ray abundance I_γ , are known, the number of atoms n of isotope Z in the sample can be calculated directly. In most cases, however, a standard is irradiated and counted

under similar conditions as the sample, and the mass of the element in the sample relative to the comparator standard is found through the equation (3) (Michael, 2008).

$$\frac{A_{sam}}{A_{srm}} = \frac{M_{sam} (e^{-\lambda t_d})_{sam}}{M_{srm} (e^{-\lambda t_d})_{srm}} \quad (3)$$

where:

A_{sam} = Activity of sample
 A_{srm} = Activity of standard reference material
 M_{sam} = Mass of element in sample (g)
 M_{srm} = Mass of element in standard reference material (g)
 t_d = Decay time

When performing short irradiations, the irradiation, decay and counting times are normally fixed, the same for all samples and standards such that the time-dependent factors cancel out. Thus equation (2) simplifies into (Michael, 2008)

$$C_{sam} = C_{srm} \frac{W_{srm} A_{sam}}{W_{sam} A_{srm}} \quad (3)$$

Where,

C_{sam} = Concentration of the element in the sample
 C_{srm} = Concentration of the element in the standard reference material
 W_{sam} = Weight of the sample and standard
 A = Activity of the sample and standard

METHODOLOGY

Sampling and sample preparation of Aphrodisiac Plants

Sample Collection and Preparation

The plants in this work (*Artemisia Absinthium* and *Boswellia serrata*) were identified at the Department of Biological Sciences of Ahmadu Bello University (ABU) Zaria and voucher specimen is kept there. Samples of the plants were collected from division of agricultural research college, ABU, Zaria Kaduna State,

Nigeria and transported in polythene bags. After collection, the samples were washed twice with tap water and rinsed with deionized water. They were first air dried and then further dried in an oven at 60°C. After drying, the samples were grounded using pestle and mortar and passed through 125µm mesh sieve.



Figure 2: Habitat, flowering twig and seeds of *Artemisia absinthium*



Figure 3: *Boswellia serrata*

Nigeria research reactor 1 (NIRR-1) which is a low-power nuclear reactor with highly enriched uranium as fuel, light water as moderator and beryllium as reflector was used for the analysis. The reactor's associated facility for radioactivity measurements is gamma ray data acquisition system. It consists of a horizontal dip-stick High Purity Germanium (HPGs) detector with a relative efficiency of 10% at 1332.4 keV gamma ray line, MAESTRO emulation software compatible with the ADCAM® multi-channel analyzer (MCA) card, associated electronic modules all made by EG & ORTEC and a personal computer.

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The efficiency curves of the detector system at near and far source detector geometries have been determined by standard gamma-ray sources in the energy range of 59.5 – 2254keV and extended to 4000keV. For data processing gamma ray spectrum analysis software WISPAN 2004 developed at CIAE, Beijing, China was used. On the basis of the well-known activation equation, the software requires that calibration factors be pre-determined by a multi-element standard reference material for elements of interest using adopted irradiation and counting regimes. The WISPAN 2004 in addition to NAA calculations, perform peak analysis, remote control of MCA and other auxiliary functions such as efficiency calibration and nuclear data generation. In this paper, the certified reference material PT19 was used to validate the results obtained. For the analysis the sample and the reference material of approximately 150 mg were weighed and wrapped in polyethylene (rabbit) capsules. The polyethylene capsules were first cleaned by soaking in 1:1HNO₃ for 3 days and washed with de-ionized water.

For irradiation, four schemes (S1, S2, L1 and L2) were adopted based on the half-life of the product radio nuclide. For elements leading to short-lived activation products (S1 and S2) the samples were each packed and sealed in 7 cm³ rabbit capsules and sent for irradiation in turn in an outer irradiation channel B4 where the neutron spectrum is “soft” the choice of the outer irradiation channel is to eliminate corrections, notable Mg in the presence of Al; Al in the presence of Si; and Na in the presence of P. This is due to the proximity of the inner channels of MNSRs to the core leading to relative higher ratio of fast-to-thermal neutron. For elements leading to long-lived activation products (L1 and L2), samples wrapped in polyethylene films were then irradiated for 6h in any of the small inner irradiation channels (i.e. A1, B1, B2 and B3) to take advantage of the maximum value of thermal neutron flux in the inner channels.

The neutron flux variability over irradiation volume was determined experimentally to be less than 2% through the measurement of specific activities of irradiated Cu wires arranged

axially and radially inside the vial. The stability of the neutron flux throughout the period of irradiation for the long irradiation was checked by monitoring the neutron flux reading of a fission chamber connected to the micro computer system.

Radioactivity measurements of induced radio nuclides were performed by the PC-based gamma ray spectrometry set up. Following the short irradiation regime the first round of counting was performed for 10min (ie.S1) after a waiting time of 2 – 15min. Samples were placed on a Plexi glass samples holder designed “H2” which corresponds to source-detector geometry of 5cm. The second round of counting was also carried out for 10min. Following the short irradiation regime (ie. S2) after a waiting period of 3 – 4 hrs. Samples were counted on a Plexi-glass holder designated as “H1” corresponding to a source-detector geometry of 1cm. The neutron flux setting for the short irradiation of the materials was raised to 5 x 10¹¹n/cm/s sensitivities for analysis of elements using procedures S1 and S2.

In the case of the long irradiation scheme, the first round of counting was carried out for 30min following the long irradiation (ie. L1) using the holder “H1” after a waiting period, counting was performed for 60min (i.e L2) after cooling time of 10 – 15days. The samples were counted using Plexi glass holder “H1”. The choice of cooling time and sample detector geometry was such that detectors’ dead time is controlled to be less than 10%. Identification of gamma-ray products of radio nuclides through their energies and quantitative analysis of their concentrations were achieved using the gamma-ray spectrum analysis software WISPAN 2004. For the biological materials the certified reference material IAEA-359 (Cabbage) and IAEA-336 (Lichen) were chosen (Jonah *et al.*, 2006).

RESULT AND DISCUSSION

The results of INAA analysis of *Artemisia absinthium* and *boswellia serrata* herbs used in this work is displayed in Table 1 along with the uncertainty values. The twenty-four elements analyzed include Mg, Al, Cl, Ca, V, Mn, Na, K, As, Br, La, Sm, U, Sc, Cr, Fe, Co, Zn, Rb, Sb, Cs, Ba,

Eu and Th. The presence of these elements in different plants was confirmed by the gamma energy of the corresponding isotope formed. In addition, these elements occur as major, minor and trace concentrations depending on the plant used for the analysis. The distribution of these elements as indicated in Table 1 below shows the unique advantage of INAA technique in terms of its element capability and ability to analyze major, minor and trace elements in a single analysis. Table 1 shows the result for two samples. It should be noted that some of the concentrations determined are below the detection limit of the INAA technique. These groups of elements are

indicated as being less than the detection limit of that particular element in Table 1.

The PT-19 SRM of biological origin, *Artemisia absinthium* and *Boswellia serrata* were analyzed for quality control and validation method. It was observed that most elemental contents are within the certified values. Standard deviations were also relatively small. An examination of the data from Table 1 shows that different plants contain many elements including Mg, Al, Cl, Ca, V, Mn, Na, K, As, Br, La, Sm, U, Sc, Cr, Fe, Co, Zn, Rb, Sb, Cs, Ba, Eu and Th in various proportions.

Table 1: Elementals concentration (in ppm) for *A. absinthium* and *B. serrata*

Elements	<i>Artemisia Absinthium</i>	<i>Boswellia Serrata</i>	Pt-19
Mg	3703 \pm 126	1670 \pm 73	2360 \pm 73
Al	69758 \pm 61	801 \pm 13	53.2 \pm 2.3
Cl	4016 \pm 60	820 \pm 27	214 \pm 13
Ca	5921 \pm 444	12350 \pm 568	15600 \pm 593
V	3.89 \pm 0.25	0.43 \pm 0.01	BDL
Mn	225 \pm 1	44.7 \pm 0.3	103 \pm 1
Na	498 \pm 2	282 \pm 1	39.5 \pm 0.6
K	40740 \pm 163	8700 \pm 78	1121 \pm 9
As	0.35 \pm 0.04	0.14 \pm 0.02	0.05 \pm 0.01
Br	4.80 \pm 0.12	2.75 \pm 0.06	1.26 \pm 0.04
La	12.8 \pm 0.08	5.62 \pm 0.06	0.72 \pm 0.02
Sm	2.01 \pm 0.01	0.62 \pm 0.01	0.127 \pm 0.003
U	0.21 \pm 0.01	0.041 \pm 0.006	BDL
Sc	1.59 \pm 0.03	0.16 \pm 0.01	BDL
Cr	6.89 \pm 0.66	1.94 \pm 0.47	BDL
Fe	1297 \pm 39	267 \pm 21	75.3 \pm 12.2
Co	0.36 \pm 0.03	BDL	BDL

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Elements	<i>Artemisia Absinthium</i>	<i>Boswellia Serrata</i>	Pt-19
Zn	57.1 ± 4.6	36.8 ± 3.6	33.9 ± 4.1
Rb	106 ± 5	BDL	9.09 ± 1.6
Sb	0.24 ± 0.05	BDL	BDL
Cs	0.82 ± 0.05	0.20 ± 0.04	BDL
Ba	89.9 ± 8.4	153 ± 30	BDL
Eu	0.06 ± 0.01	0.25 ± 0.06	BDL
Th	0.35 ± 0.01	0.064 ± 0.004	0.023 ± 0.003

The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as the mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions (Rajurkar and Pardeshi, 1997).

The active constituents of the medicinal plants are the metabolic products of the plant cells. A number of trace elements, referred to as essential elements play important role in metabolism. Deficiency of such essential elements or its concentration below a certain value always results in failure of the affected plant to grow normally and complete its life cycle (Kabata-Pendias & Pendias, 2001). An examination of the data from Table 1 shows that K content is high in all the samples. It ranges between 40740 and 8700 ppm while Na content is also high, ranging from 498 and 282 ppm. K and Na are somewhat interdependent since the regulation of potassium is intimately involved with that of sodium. Sodium is essential for regulation of osmotic pressure of the body and helps to maintain acid-base and water balance of the body (Murray et al., 2018). Its deficiency causes loss of body weight and nerves disorder.

Potassium is accumulated within human cells by the action of the Na, K-ATPase, called sodium pump and it is an activator of some enzymes and in particular co-enzyme for normal growth and muscle function (Birch and Padgham,

1994). It helps in the protein and carbohydrate metabolism. Apart from the fact that it is the principal cation of the intracellular fluid, its presence influences muscle activity particularly the cardiac muscle. Potassium deficiency causes nervous disorder, diabetes, and poor muscular control resulting in paralysis (Gropper & Smith, 2020).

The calcium content in both medicinal plants analyzed varies from 5921-12350 mg/kg with the lowest concentration from *Artemisia Absinthium* and the highest concentration from *Boswellia serrata*. Calcium is essential for healthy bones, teeth and blood because it helps to regulate the activity of skeletal muscle, heart and many other tissues (Charles, 1992). It is also required for the absorption of dietary vitamin B. additionally, it has been suggested that the synthesis of acetylcholine, an important neurotransmitter that is required for the activation of pancreatic, lipase depends heavily on the presence of Ca in appropriate concentration. Deficiency of calcium causes rickets, osteomalacia and scurvy. The recommended daily dietary allowance of Ca for children is between 500 and 1000 mg and 800 mg for adults. The extremely high Ca content in *Artemisia absinthium* and other plants analyzed in this work may be used to overcome Ca deficiency.

Iron, (Fe) is an essential element for living organisms. Its presence in the body is associated with transfer of oxygen from lungs to tissue cells and with hemoglobin in the blood cells

(Sigel, 1978). Fe deficiency mainly caused by poor dietary intake, excessive menstrual-flow or multiple births constitutes major nutritional deficiency in humans (Reddy et al; 1987) and usually results in anemia. Fe content in the various samples analyzed in this work ranges from 1297 mg/kg in *Artemisia absinthium* to 267 mg/kg in *boswellia serrata*. Therefore intake of some of these plants can readily compensate for Fe deficiency. The intake of these plants should be administered with caution though because excessive intake of Fe above the WHO recommended daily intake level of 10-30 mg/day (Cantilli et al., 1994) to avoid chronic overload of Fe in the body.

The acute chronic dose of iron in infants has been estimated to be approximately 20mg/kg and the lethal dose of about 200-300 mg/kg body weight. Intake of Fe above these lethal dosages results in hepatomegaly, cardiac disease and liver choruses (Weber, 1988). Chromium is essential in carbohydrate metabolism. It also functions in protein and cholesterol synthesis. Zinc is among the nutritionally important element (Paul et al., 2006). It is a constituent of many enzymes and insulin. Zinc is necessary for the growth and multiplication of cells skin integrity, bone metabolism and functioning of taste and eyesight (Thunus and Lejeune, 1994). Zinc is also known to prevent impaired spermatogenesis (Chaturvedi et al, 2004).

Zinc deficiency is characterized by recurrent infections, lack of immunity, growth retardation, skin changes, poor appetite and mental lethargy. Pregnant and lactating women require 20 to 25 mg, while normal adult require 15 mg of zinc every day. All the medicinal herbs analyzed contain Zn within the concentration range of 36.8 – 57.1 ppm. The lowest concentration of 36800 µg/g was found in *boswellia serrate* while the highest concentration of 57100 µg/g was found in *Artemisia absinthium*. Herbal plants containing high concentration of Zn are used in treatment of infertility, bleeding, boils, wounds, insect bites, and skin disease. Estimated safe and adequate daily intake for Zn is between 10,000 and 20,000µg/day (N.R.C, 1980). Moreover, prolonged consumption of large doses

can result in 'some health complications such as fatigue, dizziness and neutropenia (Hess et al., 2002). This may lead to the onset of diabetes. Deficiency of chromium causes insulin resistance, impairment in glucose intolerance, impaired growth and decreased respiratory quotient and as such plays an important role in diabetes treatment. Cr was determined in both the samples including *Artemisia absinthium* and *boswellia serrate* at 6.89 ppm and 1.94 ppm respectively. Therefore these plants can be explored for treatment and control of diabetes and for treatment of heart disease.

Cobalt is regarded as an essential element and form part of vitamin B12 required for red-blood synthesis. Cobalt also aid in fixing of nitrogen in the root tubercles of plants. It is widely distributed throughout the body and highest concentrations are usually found in liver, kidneys and bones. Vitamin B12 is essential for the maturation of red blood cells and normal functioning of all cells. Cobalt also plays an important role in thyroid metabolism in humans. Deficiency of vitamin B12 produces a genetic defect and anemia. The recommended daily intake of vitamin B12 for adults is 3 mg (0.13 mg Co), taking into account that only 50% is absorbed in the intestine (Thunus and Lejeune, 1994). The Co content was observed to be normal in *artemisia absinthium* with concentrations of 0.36 ppm and *boswellia serrate* that is below detection limit (BDL). Since these plants are also characterized with high Fe content, their use in medicinal preparation for treatment of anemia can be explored. It should be however be noted that there is a wide margin of safety between toxic concentration and nutritional required level of Co (Awofolu et al., 2005). However, adverse chronic effects of Co ingestion at concentration of 2mg⁻¹ may occur (DWAF, 1996).

Mn is an essential element that is normally stored in the kidney and liver in the body. Its presence in the body maintains normal bone structure, reproduction and the normal functioning of the central nervous system. It is also required for various biochemical and enzymatic processes and helps in eliminating fatigue. Its deficiency causes reproductive failure in both male and female (Guenther and Konieczynski,2003).The



high Mn content present in *artemisia absinthium* (225 ng/g) and *boswellia serrata* (44.7 ng/g) suggests that medicinal preparations involving these plants can provide Mn supplement required for various body functions (Prasad, 1993).

CONCLUSION

In conclusion the elements analyzed in *artemisia* and *boswellia* enhancers are well established. Hence, the data obtained in this study can be used to evaluate the potentiality of these plants in their use for medicinal preparations or as food supplements. The levels of the elemental concentrations obtained in the plants were seen to vary depending on the environmental conditions including soil characteristics in which the plants have grown. It is our hope that the result will serve as a reference point for agencies working with sex enhancers or interested in the elemental analysis of medicinal herbs in general.

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