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Bioinorganic elemental content of the Ghanaian aphrodisiac medicinal plant, *Paullina pinnata* Linn. (Sapindaceae)

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Macro and micro element content of dried roots sample of *Paullinia pinnata* Linn. (Sapindaceae) in Ghana were analyzed using energy-dispersive x-ray fluorescence (ED-XRF) technique. The aim was to study the possible quantitative correlation between the measured elements and the traditional usage of the plant in the treatment of sexual dysfunction or enhancing/sustaining penile erection. The analyses yielded forty-five (45) elements, of which the concentrations of four (4) elements- calcium, magnesium, potassium and zinc were significantly high. These elements are considered to play a significant role in the physiology of sexual activity or promote penile erection. They may serve as bio-markers and also support the traditional use of the roots of *P. pinnata* as an aphrodisiac in some Ghanaian communities. Heavy metals such as arsenic, cadmium, lead and mercury were also measured and quantified. The quantities of these elements were below detection limits to warrant any toxicity concerns when the plant is used as aphrodisiac.

Key words: Aphrodisiac, *Paullinia pinnata* Linn. (Sapindaceae), ED-XRF, macro and micro element, sexual activity, penile erection, heavy metals.

INTRODUCTION

Ethno medicinal usage of plants in the Ghanaian culture dates back to antiquity. There are herbal medicines often made from combinations of more than one plant species for the treatment or management of diseases such as malaria, diarrhea, dysentery, menstrual pain, waist pain and erectile dysfunction.

Erectile disorders, characterized by the inability to develop or attain and/or maintain penile erection sufficient for sexual performance (AUA, 2005), are a major health concern among men. Male sexual function,

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however diminishes with age (Enzlin et al., 2004) and life style. Aging is associated with loss of libido or impotence, which may be a resultant of various complicated conditions such as atherosclerosis, high blood pressure, diabetes, depression or medications. The prevalence rate of erectile dysfunction in Ghana is between 10 – 52% among men (Amidu et al., 2010). Besides loss of sexual function, erectile dysfunction has both negative social and psychological effects on men in the Ghanaian community, because a man's self-image and identity is associated with his sexual virility. Ghanaian males desire to stay sexually virile throughout their life. Their two main core health concerns are therefore, to maintain optimal prostate and sexual health throughout life. These they pursue through the adaptation of quasi healthy lifestyle and eating habits and use of orthodox medicines, with their associated adverse effects. The desire to avoid the adverse effects as well as high cost of orthodox medicines has resulted in the patronage of complementary and alternative therapy, including herbal medicines such as those prepared from *Paullinia pinnata*, an important option for supporting male sexual function in some communities in Ghana.

Various research activities on medicinal plants to ascertain their pharmacological activities have focused largely on the activity of secondary metabolites or organic compounds (Zamble et al., 2006; Dipankar et al., 2013; Kalimuthu and Prabakaran, 2013). Few of these studies have examined the macro/micro elements and their physiological effects although a number of them have significant impact on human health. Some of these elements are vital in numerous metabolic functions in the human body (Prasad, 2008). Their biological significance is dependent on their concentration and their physiological interactions. For example, micro/macro elements such as zinc, selenium, magnesium, potassium and calcium, are known to be involved in the physiological processes that promote penile erection (Jeon, 2005; Ghofrani et al., 2006; Adrogue and Madias, 2007; Mike, 2013). Levels of magnesium and zinc in the prostate are known to be high in seminal fluid and assist in male fertility (Zieve and Chen, 2010). Zinc on the other hand is reported to stimulate the pituitary glands to release hormones that stimulate testosterone production (Myatt, 2012). Similarly, the M site of the phosphodiesterase-5 enzyme, implicated in erection sustenance, is reported to contain zinc and magnesium ions (Jeon, 2005).

According to reports, magnesium facilitates production of androgen, estrogen and neurotransmitters that regulate sex drive and hence acts as an efficient testosterone enhancer (Myatt, 2012). Potassium is also implicated in vasodilation due to hyperpolarization of vascular smooth muscle (Ghofrani et al., 2006). Reports indicate that 50% of selenium found in the testicles and seminal ducts of males is required for healthy sperm production (Myatt, 2012). Calcium ions also stimulate neuronal nitric oxide synthase through calmodulin path-way to affect erectile function.

Roots of the traditionally acclaimed Ghanaian aphrodisiac plant, *P. pinnata* (Linn) (Sapindaceae) – also known as Sweet gum or "Toantini" (among the Akans in Ghana), a woody or sub-woody climbing plant, commonly found in secondary forests in Ghana (GHP, 2007) have been used traditionally not only as an aphrodisiac among other uses (Annan et al., 2013, Chabra et al., 1991; Gill, 1992). In view of the important physiological roles of micro/macro elements in sexual function, we sorted to profile *P. pinnata* roots samples for these elements with a view of establishing whether they may be either physiologically or pharmacologically significant in managing erectile dysfunction.

EDXRF was used to analyze the roots samples for micro and macro element contents. Instrumental analytical methods used to qualify and quantify micro and macro elements in plants include neutron activation analysis (NAA), optical emission spectroscopy (OES), atomic absorption spectroscopy (AAS), mass spectroscopy (MS), inductively coupled plasma mass spectroscopy (ICPMS) and total reflection x-ray fluorescence (TXRF). Energy dispersive x-ray fluorescence is important to biologist, environmentalist, geologist, clinician, pharmacologist, biochemist and drug regulatory bodies for quality control. This is due to the non-destructive nature of analysis, rapid, sensitive, relatively cost effective sample analysis and its potential to enable simultaneous, qualitative, semi-quantitative and quantitative analysis without chemical pretreatment of samples of any size or number (Anzelmo and Lindsay, 1987; Jenkins et al., 2000; Ashok, 2014; Metz et al., 1994; Revenko, 2002; Sieber, 2000). This has opened doors for many research works on plant tissues by different researchers (Margui et al., 2005; Vazquez et al., 2003).

MATERIALS AND METHODS

Sample collection and preparation

Roots of *P. pinnata* were collected from Sekyere-Kwamang in the Ashanti region, Ghana. These were washed and room dried for two weeks. The roots were soaked in liquid nitrogen (-346 and -320.44°F, that is, 63 – 77K) for 10 min, powdered and sieved with a mesh size (aperture) of 180 µm into a fine powder and kept in a dry well-labelled container. Plant cells easily disintegrate if pretreated with liquid nitrogen and can be easily milled or blended into powder (Tim, 2014). Before pelletation, the sample was kept in an oven at 60°C overnight. Due to their morphology and the loose nature (Queralt et al., 2005), triplicate weighed samples – 4000 mg/sample - were added separately to 900 mg Fluxana H Elektronic BM-0002-1 (Licowax C micropowder PM-Hoechstwax) as binder, the mixture was homogenized using the RETSCH Mixer Mill (MM301) for 3 min and pressed manually with SPECAC hydraulic press for 2 min with a maximum pressure limit of 15 tons (15000 kg) into pellets of 32 mm in diameter and 3 mm thickness for subsequent XRF measurements. Time between pelletation and measurement was kept short to avoid deformation of the flat surfaces of the pellets (Anjos et al., 2002). Spectro X-Lab 2000 spectrometer (Geological Survey Department, Accra, Ghana) enhanced with three-axial

Table 1. Content of selected macro elements in *P. pinnata* (mg/4000 mg sample).

Element	Mean	Content (%)	CV (%)
Mg	15.99 ± 1.12	0.40	7.00
Al	7.66 ± 0.02	0.19	0.31
Si	14.10 ± 0.34	0.35	2.39
P	3.68 ± 0.12	0.09	3.35
Cl	1.55 ± 0.03	0.04	2.10
K	31.27 ± 0.82	0.78	2.63
Ca	101.83 ± 3.49	2.55	3.43
Ti	0.34 ± 0.04	0.009	12.46
Mn	0.25 ± 0.02	0.006	8.72
Fe	2.89 ± 0.14	0.07	4.89

CV: Coefficient of variance.

Table 2. Content of micro elements in *P. pinnata* (mg/4000mg sample).

Element	Mean	Content (%)	CV (%)
Cr	0.05 ± 0.01	0.001	24.81
Ni	0.01 ± 0.00	0.0003	2.94
Cu	0.03 ± 0.00	0.0008	9.23
Zn	0.03 ± 0.00	0.0007	12.37
As	<0.4	NQ	NQ
Rb	0.02 ± 0.00	0.0006	3.33
Sr	0.65 ± 0.01	0.02	2.12
Ba	0.11 ± 0.02	0.003	18.39
Cd	ND	ND	ND
Se	ND	ND	ND
Hg	ND	ND	ND

CV: Coefficient of variance; NQ – not quantified; ND – not detected.

geometry to reduce background noise due to radiation polarization and its monochromatic radiations emitted from the x-ray tube to excite the atoms of the samples was used for simultaneous analysis and measurement of the elemental content of the samples. This spectrometer is equipped with Rh anode and 400W Pd x-ray tube, a 0.5 mm Be end window tube, a Si (Li) detector (resolution of 148 eV – 1000 cps Mn Kα), available targets (Al_2O_3 and B_4C used as a BARKLA polarizer), an HOPG (High Oriented Pyrolitic Graphite) as a BRAGG polarizer, Al, Mo and Co as secondary target and a 0.5 mm Be side window. It has a carousel (circular rotating sample changer) inside a sample chamber with a capacity of 20 sample holder disc (32 mm) for sequential sample analyses. The radiation chamber was cooled using liquid nitrogen. Its computer-based multi-channel analyzer– SPECTRO X-Lab Pro Software package (Turboquant) controlled and computed spectral analysis, collected, evaluated and stored data. Combination of these different targets gave a typical detection limit for light elements (Si, Al, Mg and Na) in the range of 25-50 ppm. For heavy metals, 1-5 ppm were the limits of detection. The spectrometer was factory calibrated using a number of international rock standards.

RESULTS AND DISCUSSION

A total of forty-five (45) micro and macro elements were detected with the ED-XRF. From these, twenty-two (22) elements, eleven (11) macro and eleven (11) micro elements were identified and quantified. The macro elements as presented in Table 1 were sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), potassium (K), chlorine (Cl), calcium (Ca), titanium (Ti), manganese (Mn) and iron (Fe).

The microelements in Table 2 were chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), rubidium

Table 3. Levels of selected elements implicated in the aphrodisiac potential of *P. pinnata.*

Element	Mean (mg/4000 mg sample)	Content (%)	CV (%)
Mg	15.99 ± 1.12	0.40	7.00
K	31.27 ± 0.82	0.78	2.63
Ca	101.83 ± 3.49	2.55	3.43
Zn	0.03 ± 0.00	0.0007	12.37

CV: Coefficient of variance.

Table 4. Levels of some toxic heavy metals detected in *Paullina pinnata* (%/4000 mg sample in ppm).

Element	A_1	A_2	A_3
As	<0.4	<0.4	<0.4
Cd	<0.9	<0.8	<0.7
Hg	<0.5	<0.9	<0.9
Pb	<0.8	<0.8	<0.8

(Rb), strontium (Sr), barium (Ba), cadmium (Cd), selenium (Se) and mercury (Hg).

Elements which are implicated in the physiology of male penile erection evaluated in this study were Mg, Ca, K, and Zn listed in Table 3. Though selenium in Table 2 is reported to assist in the production of healthy sperms, the value recorded was below detectable limit.

The four gram (4g) samples were converted into milligrams (mg). Simple statistics (mean, standard deviation and coefficient of variation - CV) of the results were calculated to gain a better understanding of the results. The CV, that is, standard deviation versus mean ratio, expressed as a percentage, is reported to be a better way to express the goodness of a variable to be used for quality control and classification in environmental systems are shown in Tables 1, 2 and 3 (Queralt et al., 2005).

It could be observed from Table 3 that the levels of Ca, Zn, Mg and K in the four gram samples were prominent. Quantities of these elements may be sufficient as adjuvants in effecting penile erection, sustenance or triggering sexual desire in males. These observed levels may therefore support the traditional usage as an aphrodisiac.

The recommended average daily allowance for calcium is given as 1300 mg/day and the upper tolerable intake as 2500 mg/day (DRI, 2004). The high concentration of Ca (101.83 mg/4000 mg) in plant sample may account partly for its aphrodisiac effect. Ca^{2+} ion concentration plays a prominent role in Ca^{2+} – calmodulin pathway in the initiation and sustenance of erectile function (Tom, 2000). Literature reviews point to potassium as a vasodilator, inducing hyperpolarization of vascular smooth muscle thereby potentiating erectile function in males (Adrogue and Madias, 2007).

The recommended daily intake of potassium is 2300 mg/day (DRI, 2004). Thus, the relatively high concentration of K in *P. pinnata* (31.27 mg/plant material) may be associated with the aphrodisiac effect of the plant.

Magnesium is implicated in the production of androgen, estrogen and neurotransmitters effecting sexual drive (Edor et al., 2003). The 15.99 mg/ concentration of Mg detected in the plant material, though in a relatively smaller quantity, cannot be overruled to enhance levels of neurotransmitters and hormones through various mechanisms to help enhance sex drive and hence the aphrodisiac action of *P. pinnata*.

Though 0.03 mg/4000 mg concentration of zinc is recorded in plant sample, Zn is required for proper testosterone and sperm development (Yates, 2013). The prostate glands stores Zn in high concentrations and low concentrations are found in men with erectile difficulties. It also causes stimulation of the pituitary glands to release hormones responsible for testosterone production.

Selenium though below detectable limits, about 50% of selenium in males is said to be found in the testicles and seminal ducts. Reduction or loss of selenium levels contribute to low sperm count which may lead to erectile dysfunction (Mike, 2013).

Arsenic, cadmium, lead and mercury values recorded are shown in Table 4 were below detectable limits. The site of sample collection was in a forest, far from regular anthropogenic activities and this may account for the observed results (WHO, 2008).

Conclusion

Elements like Ca, Zn, Mg, Se and K are essential for penile erection and/or sustenance. The results indicated that the quantities implicated in penile erection or sustenance was within recommended levels except selenium.

Arsenic, cadmium, mercury and lead even below detectable limits, may pose as a threat to human health if consumption is not controlled.

XRF, aside being non-destructive, rapid, sensitive, relatively cost effective measurement, is suitable for simultaneous qualitative, semi-quantitative and quantitative analysis without prior chemical pretreatment of samples irrespective of the size and number.

The levels (concentrations) of micro and macro elements in the roots of *P. pinnata* in this study support the aphrodisiac use of the plant in the management of erectile disorders in Ghana. These micro and macro elements may serve as bio-markers and are recommended for analysis of plants used as aphrodisiacs.

Conflict of interest

This is an independently prepared paper. Authors have not declared any conflict of interest either commercially or otherwise in the publication of this article.

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