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Determination of the Concentrations of Zinc and Vitamin C in Oysters and Some Medicinal Plants used to Correct Male Factor Infertility

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ABSTRACT

Zinc and vitamin C have been found to be essential for male factor fertility. In this study we report the concentrations of zinc and vitamin C in several plants which are being used in herbal medicine to correct male factor infertility. Some of these, namely, *Momordica charantia*, *Newbouldea laevis* (leaves) and *Rauvolfia vomitoria* (leaves) were found to have high concentrations of both zinc and vitamin C which indicate that there is a scientific basis for their use in correcting male factor infertility. The other plants studied were found to be rich in either zinc or vitamin C. Some of the plants may promote fertility by treating some underlying diseases such as cancer which may impair fertility.

Keywords: Zinc and Vitamin C; Profertility plants; Oysters; Male factor infertility.

INTRODUCTION

Male factor infertility has been estimated to account for approximately 50% of all problems with fertility. In 1% of patients, a significant medical condition such as cancer of the testis, brain tumor or spinal cord tumor was identified as the cause of male infertility (Honig, et al., 1994). Reduced sperm count and poor sperm quality can also be responsible for male factor infertility. In fact most cases of infertility are due to abnormal sperm count or low sperm motility. Zinc and vitamin B complex are critical nutrients in the male reproductive system for proper hormone metabolism, sperm formation and motility. A number of nutritional therapies have been shown to improve sperm counts and sperm motility. These include carnitine, arginine, zinc, selenium and vitamin B-12 as well as antioxidants such as vitamin C, vitamin E, glutathione and coenzyme Q-10 (Sinclair, 2000). A zinc deficiency can cause chromosome changes in sperm leading to reduced fertility and an increased risk of miscarriage. Deficiencies in zinc can lead to impotence and it is known that zinc may improve sexual performance (Takahara, et al., 1987). Zinc levels are usually lower in infertile men with diminished sperm count and studies have found supplemental zinc may prove helpful in treating male infertility (Madding, et al., 1986). Reactive oxygen species such as free radicals may reduce sperm count, quality and motility. Vitamins C and E are powerful antioxidants and vitamin E has been found to increase fertilization rates significantly while vitamin C enhances sperm quality and prevents sperm agglutination thus making them more motile with more forward progression (Glenville, 2008; Geva, et al.,

1996; Dawson, et al., 1992). Vitamin C is a water-soluble vitamin which is readily extracted in aqueous medium. It has been found to help in preventing cell damage by neutralizing free radicals (Fraga, et al., 1991).

In this study we report the concentrations of zinc and vitamin C in several plants used in herbal medicine to promote fertility in male human subjects in Nigeria and the sub-region. These include the leaves and roots of *Annona senegalensis*, the leaves and roots of *Costus afer*, the stem of *Cissus populnea*, the seeds of *Garcinia kola*, the whole plant of *Momordica charantia*, the leaves and roots of both *Newbouldea laevis* and *Rauvolfia vomitoria*. Oyster has been found to be rich in zinc and was included in this study as a reference.

A. senegalensis has several medicinal properties. The roots, bark, leaves and seeds are used as herbal medication for treating cancer, dysentery, gastroenteritis, snakebite, guinea worms, cough, venereal diseases, tooth-ache, eye infections, respiratory infections and tumor growth (Neuwinger, 1996). Venereal diseases and intestinal disorders are treated with preparations of the roots (Sofowora, 1993). The leaves have been found to contain rutin, quercetin, quercitrin while the bark contains alkaloids, saponins and tannins (Potchoo, et al., 2008).

C. populnea has a number of medicinal uses in different parts of the world. In Benin Republic, it is used for its diuretic properties while in Ghana it is used as a post-harvest ethnobotanical protectant (Belmain, et al., 2000). The aqueous extract of the stem bark is used as a fertility enhancer in males in South Western Nigeria (Ojekale, et al., 2006). Studies on the freeze-dried extract of *C. populnea* on rats showed that sperm count was significantly ($P < 0.05$) increased by 37%. The serum Lutenizing Hormone (LH) and Follicular Stimulating Hormone (FSH) were elevated by 11% and 29% respectively (Smith, et al., 2002). Phytochemistry of the stem bark of the plant showed the presence of tannins, flavonoids, saponins and steroids (Ojekale, et al., 2006). These compounds are associated with functions related to fertility enhancement potentials (Das, et al., 2004) Extracts from the plant have also been used for treating urinary tract infections (Ojekale, et al., 2007). The anti-sickling activities of extracts of the roots on HbS red blood from sickle-cell patients have also been confirmed. The same study also reports the presence of anthraquinone derivatives, steroid glycosides as well as cardiac glycosides and the absence of alkaloids and tannins (Moody, et al., 2003).

C. afer is used for the treatment of cough, diabetes, rheumatic swellings, cuts, wounds, malaria, jaundice, gonorrhoea and bilharzias. It also functions as diuretic and anti-snake venom (Odugbemi, 2008). A decoction of the dried plant is used for the treatment of various eye infections, headache, fever and rheumatism (Bouquet and Der bray, 1974). In Nigeria the pounded fruit is used as a relief for cough (Dalziel, 1937), while the sap is taken for malaria and to clear urine. An infusion of the roots is taken for stomach-ache and is considered to be a stimulant and an aphrodisiac (Ainslie, 1937).

Various parts of the *G. kola* plant, namely seeds, root, stem-bark and fruits are used in medicinal preparations for treating dysentery, chest pain, body weakness, stomach pain, glaucoma, cough, fever, toothache, throat and respiratory ailments, liver disorders, headache, evacuent and snake bite. It is also used as anti-cancer medication and as an aid to boost sperm count (Odugbemi, 2008). The bark and leaves are administered to men in Sierra Leone to enhance sexual potency. The seeds are said to increase male potency when chopped up and steeped in water or palm wine and ingested. The seeds have also been reported to possess antimicrobial, anti-inflammatory and antiviral properties (Idika and Niemogha, 2008). An ethnobotanical survey of medicinal plants used in the management of diabetes mellitus conducted in South Western Nigeria identified the seeds of *G. kola* and the roots of *M. charantia*, as being helpful in managing diabetes mellitus (Abo, et al., 2008). The anti-diabetic activity of *G. kola* has also been reported (Iwu, et al., 1990).

M. charantia is used as an anthelmintic, antimicrobial and aphrodisiac (Odugbemi, 2006; Ainslie, 1937). When used as aphrodisiac, the leaf sap is added to a calabash of palm-

wine which is drunk during the course of the day. The roots are also used as aphrodisiac (Weniger, et al., 1986). The sap from the leaves is used to correct impaired vision in Southern Nigeria (Ogunlesi, et al., 2008). *M. charantia* whole-plant aqueous extract has been demonstrated to possess hypoglycaemic and hypotensive properties thus lending pharmacological credence to its ethnomedical application in the control of diabetes mellitus and hypertension among Africans (Ojewole, et al., 2006). The hypoglycaemic activity of the plant has been demonstrated in diabetic models (Bailey, et al., 1985). The leaves and fruits are used as an antihelmintic and purgatives, and for treatment of diabetes mellitus.

The leaves of *N. laevis* are used as aphrodisiacs. Other parts of the plant are used for treating several ailments and diseases including round worms, dysentery, elephantiasis, convulsions, malaria, cough, migraine, stomach-ache, yellow fever, infertility and ear-ache (Odugbemi, 2008). The leaves are mixed with other plants to give a mixture whose extracts are used to treat fibroids (Fabeku and Akinsulire, 2008). The stem bark is used for treating various skin infections while the leaves are used to treat conjunctivitis (Zailani and Ahmed, 2008). A study on the in-vitro assessment of the antioxidant activity of *N. laevis* showed that the 95% methanolic extract of the dried stem bark achieved a maximum antioxidant activity of 4% within 40 min (Ogunlana and Ogunlana, 2008). *N. laevis* has also been shown to have strong anti-candida activity (Hoffman, et al., 2004). Preliminary phytochemical screening of the methanolic leaf extract in Nigeria revealed the presence of flavonoids, tannins, terpenes, steroidal and cardiac glycosides. The extract inhibited the growth of several microorganisms (Usman and Osuji, 2007). A study of the chemical constituents of the roots of *N. laevis* revealed the presence of fifteen compounds including a naphthoquinone-anthraquinone coupled pigment which is a potential antimalarial and antimicrobial (Eyong, et al., 2006). Other isolates include antifungal and antibacterial naphthoquinones, phenylpropanoid glycosides from the methanolic extract of the roots (Gafner, et al., 1997) and a naphthoquinone from the dichloromethane extract of the roots (Gafner, et al., 1998).

Various parts of *R. vomitoria* such as the roots, leaves and stem bark have been reported to be useful in the treatment of hypertension, insomnia, nervous disorder, jaundice, diarrhoea, scabies and malaria (Odugbemi, 2006). The plant contains over 50 alkaloids such as rauwolfine, reserpine, rescinnamine, serpentine, ajmaline, serpentinine, steroid-serposterol and saponin (Iwu and Court, 1982). The parts mostly used are the roots and leaves. A bioactive β -carboline alkaloid, alstonine, present in the roots and leaves has been shown to have anti-cancer activity (Demis, et al., 2006). The alkaloid, reserpine, is useful for the treatment of hypertension. Folk medicinal uses of the roots are extensive, particularly for their aphrodisiac, emetic, purgative, abortive and insecticidal properties (Principe, 1989). Chopped leaves stewed with animal fat are applied to swellings (Burkill, 1994).

MATERIALS AND METHODS

Collection of samples: Batches of plant materials were collected between January and April 2005 from Mushin Market, Lagos. The plants were identified by Dr. A.A. Adekunle of the Department of Botany and Microbiology, University of Lagos and Mr. T. K. Odewo formerly of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where vouchers were deposited and herbarium numbers were issued. These are included in Table-1. Samples collected between the dry season, January-March are referred to as January batch and those collected in April are referred to as the April batch.

Determination of the Concentrations of Zinc and Vitamin C: The samples were air-dried at room temperature and pulverized just before digestion for determination of zinc or extraction with 0.5% oxalic acid for the determination of vitamin C.

Determination of zinc was according to the procedure in Official Methods of Analysis of AOAC (AOAC, 1980) using 2 g of the freshly powdered sample. Measurements were carried out by AAS on 200A Buck Scientific Spectrophotometer. For determination of vitamin C, 2 g of the freshly powdered sample was homogenized with 100 cm³ 0.5% oxalic acid

acid, filtered through celite filter and the filtrate made up to 100 cm³ with 0.5% oxalic acid. The liquid obtained was filtered and aliquots titrated with freshly prepared 2, 6-dichlorophenol indophenol. Ascorbic acid was used for calibration. All determinations were carried out using duplicate samples. Titrations were carried out in triplicates and the mean of each set was used to calculate the concentration of vitamin C.

RESULTS

The results for the concentrations of zinc and vitamin C in the January and April batches of the samples are reported in Table-1. The zinc concentrations range from 0.88 mg/100 g in the roots of *A. senegalensis* to 6.20 mg/100 g in *M. charantia* sample among the plant materials under investigation. The concentrations of vitamin C range from 11.20 mg/100 g in the seeds of *G. kola* to 41.20 mg/100 g in *M. charantia*.

DISCUSSION

The plants under investigation are rich in zinc since the concentrations of zinc in them compare very well with those of zinc-rich foods of plant origin such as mushrooms, green peas (boiled) and sesame seeds, in which the zinc levels are in the range of 0.80-4.01 mg/100 g sample (IZiNCG, 2004). Therefore they are likely to promote male fertility arising from zinc deficiency. The results show that the zinc concentrations are season-dependent in *R. vomitoria* (leaves and roots) and *C. afer* (roots) which have higher zinc concentrations in January which is in the dry season than in April when the rains have begun. The oysters obtained in January were markedly more robust than those available in April and the difference in maturity possibly accounts for the appreciable difference in their zinc concentrations. *G. kola* is usually available in the dried forms and hence the time of purchase may not reflect the time of harvesting, hence it may be difficult to draw any conclusion on the seasonal dependence of the zinc concentrations in them. In two of the plants, namely *A. senegalensis* and *N. laevis* the zinc concentrations appear to be appreciably more in the leaves than in the roots in the batches analyzed.

The concentrations of vitamin C in the plants range from 11.2-41.2 mg/100 g sample determined as ascorbic acid. Leafy plant parts which are rich sources of vitamin C include broccoli (90 mg/100 g sample), brussels sprouts (80 mg/100 g sample), cauliflower (40 mg/100 g sample) and spinach (30 mg/100 g sample) (Vitamin C, Wikipedia). In the list of plant materials investigated, only four plant parts have vitamin C concentrations above 30 mg/100 g sample. These are *M. charantia*, *N. laevis* (April batch of leaves and roots) and *R. vomitoria* (April batch of leaves and roots).

From the concentrations of zinc and vitamin C, it could be concluded that the plants, *M. charantia*, *N. laevis* (leaves), *R. vomitoria* (leaves) and *N. laevis* (roots) would be effective in improving male fertility arising from zinc and vitamin C deficiency. The leaves of the April batch of *A. senegalensis* are moderately rich in both zinc and vitamin C which would increase the testosterone production, sperm quality and motility and thus enhance male fertility (Sinclair, 2000).

The plants under investigation have been proved scientifically to be effective in the treatment of various diseases. For example, *A. senegalensis* has been found to contain quercetin (Potchoo, et al., 2008) which is useful in the treatment of tumors, cancer, infections and venereal diseases (Neuwinger, 1996). Tumors in certain parts of the male anatomy and venereal diseases have been found to impair fertility (Honig, et al., 1994; Greendale, et al., 1993). Thus ingestion of extracts of the plant may reduce tumors and/or eliminate venereal diseases in affected subjects thereby leading to improved fertility.

We can summarize as follows. Some of the plants such as *M. charantia* and *N. laevis* have been demonstrated to have antimicrobial activity (Odugbemi, 2006; Gafner, et al., 1997). *A. senegalensis* is used for the treatment of venereal diseases (Sofowora, 1993) and *C. populnea* has been found effective in the treatment of urinary tract infection (Ojekale, et al., 2009).

al., 2007). The claim in Benin Republic that it is effective as diuretic may render the plant as an anti-hypertensive agent and the relief of hypertension may result in improved fertility. The steroids present in the plant may be responsible for its spermatogenic activity (Ojekale, et al., 2006) and hence will promote male fertility arising from oligospermia. *C. afer* is useful for the treatment of various eye infections (Bouquet and Der bray, 1974) and hence may have antimicrobial activity. *G. kola* seeds have been found to have strong antimicrobial activity (Adedeji, et al., 2006). They have also been found to promote testosterone production (Braide, et al., 2003; Akpantha, et al., 2005). The seeds also contain flavonoids and steroids (Ojekale, et al., 2006) which are associated with functions related to fertility enhancement (Das, et al., 2004). These listed constituents and bioactivities can serve as promoters of fertility in male subjects. *M. charantia* is rich in zinc and vitamin C, thus ingestion of the plant will enhance sperm production, quality and motility. Zinc is also essential for the production of adequate quantities of testosterone. Thus zinc-rich plants will enhance fertility in males (Honig, et al., 1994; Takihara, et al., 1987; Glenville, 2008; Dawson, et al., 1992). Vitamin C is an antioxidant and such compounds have been found to enhance sperm quality and prevent sperms from agglutination thus making them more motile with forward progression (Glenville, 2008; Dawson, et al., 1992). The antihypertensive and antidiabetic properties of the plant extract on rats have been established (Ojewole, et al., 2006) and correction of hypertension and diabetes may result in enhanced fertility.

The results of this study indicate that the leaves of *N. laevis* are rich in zinc and vitamin C while the roots are rich in vitamin C but contain relatively moderate quantities of zinc. Flavonoids, steroidal glycosides and antioxidants have been reported present in the methanolic leaf extract of *N. laevis* (Usman and Osuji, 2007). These compounds promote fertility (Das, et al., 2004; Glenville, 2008; Geva, at al., 1996). The January samples of the leaves and roots of *R. vomitoria* are rich in zinc while the April samples are rich in vitamin C. Thus the January batch may be more effective than the April batch as fertility enhancers where zinc therapy is required. The leaves when processed are used to reduce swellings (Burkill, 1994). Swellings on some organs in the male anatomy may reduce fertility. The roots and leaves have been reported to contain a bioactive alkaloid which has anti-prostrate cancer activity (Demis, et al., 2006). Some types of cancers have been identified as the cause of male infertility (Honig, et al., 1994). Extracts having anti-diabetic activity have been obtained from the leaves, stems and roots of the plant (Chevallier, 1996). Such extracts may serve as fertility enhancers where infertility is linked to such disorders.

Thus several of the plants under investigation possess medicinal properties in addition to zinc and vitamin C and ingestion of such plant parts can enhance male fertility. *M. charantia*, *N. laevis* (leaves) and *R. vomitoria* (leaves) in addition to the high levels of zinc and vitamin C present have been proven to possess various components, stated previously, which can promote fertility in male subjects. *A. senegalensis*, (leaves and roots), *C. populnea* (stem) and *G. kola* (seeds) though less rich in one or both of zinc and vitamin C can treat some of underlying diseases which impair fertility.

CONCLUSION

Some of the plants, namely, *M. charantia*, *N. laevis* and *R. vomitoria* have high concentrations of both zinc and vitamin C and thus may enhance fertility in male subjects. Several of these plants have been shown to treat several diseases which may impair fertility in male subjects.

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Table-1: Concentrations of zinc and vitamin C in some medicinal plants used in treating male infertility.

	Botanical Names, Plant parts and Herbarium Numbers	Common Names	Zn (mg/100 g sample)		Vitamin C (mg/100 g sample)	
			January batch	April batch	January batch	April batch
1.	<i>Anona senegalensis</i> (leaves) FHI 107755	Wild custard apple, Abo (Yor.), Uburu Ocha (Igbo), Tallafa Maraayu (Hausa)	3.05	4.28	22.50	20.50
2.	<i>Anona senegalensis</i> (roots) FHI 107755	Wild custard apple, Abo (Yor.), Uburu Ocha (Igbo) Ginger lily, Tete-egun (Yoruba) Okpete (Igbo) Kuku zuwa (Hausa), Tallafa Maraayu (Hausa)	2.09	0.88	22.00	16.20
3.	<i>Cissus Populnea</i> (Stem) FHI 108222	Ogbolo (Yoruba) Daafaraa (Hausa)	2.78	2.87	16.2	20.00
4.	<i>Costus afer</i> (leaves) FHI 107754	Ginger lily, Tete-egun (Yoruba) Okpete (Igbo) Kuku zuwa (Hausa)	4.75	4.09	15.00	15.50
5.	<i>Costus afer</i> (roots) FHI 107754	Ginger lily, Tete-egun (Yoruba) Okpete (Igbo) Kuku zuwa (Hausa)	5.57	3.65	12.50	16.20
6	<i>Garcinia kola</i> (seeds) FHI 107758	Bitter cola, orogbo (Yoruba) Ugolu (Igbo) Ciidia goro (Hausa)	2.44	1.27	11.20	11.20
7	<i>Momordica charantia</i> (whole plant) FHI 107759	African cucumber, Ejirin (Yoruba) Kakaye (Igbo)	6.20	5.31	31.30	41.20
8	<i>Newbouldea laevis</i> (leaves) FHI 107753	Indian Cork tree, Fertility plant, Akoko (Yoruba) Umune (Igbo) Aduruku (Hausa)	5.41	5.46	28.80	37.50
9	<i>Newbouldea laevis</i> (roots) FHI 107753	Indian Cork tree, Fertility plant, Akoko (Yoruba) Umune (Igbo) Aduruku (Hausa)	3.86	3.65	28.80	35.00
10	<i>Rauvolfia vomitoria</i> (leaves) FHI 107757	Swizzle stick, Asofeyeje (Yoruba) Akanta (Igbo) Wada (Hausa)	5.29	3.32	17.50	35.00
11	<i>Rauvolfia vomitoria</i> (roots) FHI 107757	Swizzle stick, Asofeyeje (Yoruba) Akanta (Igbo) Wada (Hausa)	4.41	2.56	13.80	32.00
12	Oyster		55.71	28.82	28.80	30.00