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# Lipid Peroxidation as Index of Activity in Aphrodisiac Herbs

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**Abstract:** ROS are important mediators of sperm function. Production of malondialdehyde (MDA), an end product of LPO, has been reported in spermatozoa. Ethnobotanical survey revealed [*Anthocleista djalonensis* A. Chev (Loganiaceae), *Carpolobia lutea* G. Don (Polygalaceae), *Cassia sieberiana* DC (Leguminosae), *Chasmanthera dependens* Hochst (Menispermaceae), *Cissus populnea* Guill and Perr (Vitaceae), *Cnestis ferruginea* DC (Connaraceae), *Dioscorea cayenensis* Lam. (Dioscoreaceae), *Lecaniodiscus cupanioides* Planch (Sapindaceae) and *Microdermis keayana* J. Leonard (Pandaceae)] as common medicinal plants used locally in South West Nigeria to boost libido, induce erection, increase sperm count and consequently male fertility. Inhibition of lipid peroxidation by these plant extracts as index of male fertility was assessed. MDA was assayed by thiobarbituric acid (TBA) reaction on lipid peroxidation in raw/cooked fish homogenates and measured as the amount of thiobarbituric acid reactive Sample (TBARS) in nmol $mg^{-1}$ tissue. Extracts significantly inhibited extent of lipid peroxidation. *A. djalonensis* was most active while *D. cayenensis* was least active in both raw and cooked fish homogenates. TBARS activity/Concentration ($R^2 = 0.9533$ for raw and $R^2 = 0.9739$ for cooked). Thus these plants may be considered as cheap and readily available sources of treating sexual dysfunction in men.

**Key words:** Ethnobotany, aphrodisiac herbs, fidelity index, lipid peroxidation, rapid assay, index of activity

## INTRODUCTION

Infertility affects approximately 15% of all couples trying to conceive. Male infertility is the sole or contributing factor in roughly half of these cases and no identifiable cause can be found in over 25% of infertile males. There are many natural herbal and nutritional aphrodisiacs that enhance sexual drive and pleasure in both men and women, along with increase libido, improve sexual performance, blood flow, increase force and intensity of ejaculation. Clinical studies have validated that certain herbs do indeed have aphrodisiac activity. They also have anabolic and growth hormone stimulating properties (Iwu, 1993; Smith *et al*., 2002; Tajuddin *et al*., 2003; Rajeshwar *et al*., 2005; Leslie, 2006).

Excessive production of free radicals or Reactive Oxygen Species (ROS) can damage sperm and ROS have been extensively studied as one of the mechanisms of infertility. Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major ROS present in seminal plasma. Cells living under aerobic conditions constantly face the oxygen ($O_2$) paradox-$O_2$ is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival, or both; hence, any excess ROS must be continuously inactivated in order to maintain normal cell function. This function is taken up by the antioxidants present in the seminal plasma. When there is an excessive production of ROS

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or impaired antioxidant defense mechanisms, Oxidative Stress (OS) occurs, which is harmful to spermatozoa (Dandekar *et al*., 2002).

Mammalian spermatozoa are rich in (Poly Unsaturated Fatty Acids-PUFA) and are very susceptible to attack by ROS and membrane lipid peroxide ion. Normally, a balance is maintained between the amount of ROS produced and that scavenged. Cellular damage arises when this equilibrium is disturbed. A shift in the levels of ROS towards pro-oxidants in semen and vaginal secretions can induce an oxidative stress on spermatozoa (Dandekar *et al*., 2002). Theoretically, cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. The scavenging potential in gonads and seminal fluid is normally maintained by adequate levels of antioxidants superoxide dismutase (SOD), catalase and probably glutathione (GSH) peroxidase and reductase (Sikka *et al*., 1995). Synthesis of NO in response to infection and inflammation could contribute to poor sperm motility and function and may lead to infertility (Rosselli *et al*., 1995). Nitric oxide-induced toxicity is also mediated indirectly through its interaction with superoxide anions and formation of peroxynitrite anion, which when protonated, decomposes to form OH-and $NO_2$, both of which are cytotoxic agents (Beckman *et al*., 1990).

Mammalian spermatozoa susceptibility to ROS attack results in a decreased sperm motility, presumably by a rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability and increased midpiece morphology defects with deleterious effects on sperm capacitation and acrosome reaction (Lamirande and Gagnon, 1992). Lipid peroxidation of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to infertility (Alvarez *et al*., 1987).

Lipid peroxidation plays a significant role in the etiology of defective sperm-function. The onset of lipid peroxidation in susceptible forms leads to the progressive accumulation of lipid hydroperoxides in the sperm plasma membrane, which decomposes to form malonaldehyde (MDA) which is an index of lipid peroxidative damage.

Swelling of sperms is indicative of normal sperm function and transport of molecules across the sperm membrane. Loss of sperm-membrane fluidity due to initiation of lipid peroxidation may cause oxidation induced cellular injury to the spermatozoa. Lipid peroxidation also impairs cell membrane ion-exchange that is essential for maintaining normal sperm-motility (Dandekar *et al*., 2002).

Free radical mediated oxidation of lipids particularly affects the PUFA due to their high degree of unsaturation. PUFA's present in fish oils because of their high degree of unsaturation, have increased vulnerability to lipid peroxidation. To counteract this vulnerability, the practice of adding antioxidants to fish oils has come in vogue. It has been shown that supplementation of fish oil with antioxidant Vitamin E inhibits peroxidation of oil (Godwin and Prahbu, 2006).

Both natural and synthetic antioxidant products have gained attention by the cosmetic, nutritional and pharmaceutical industries. How ever, their usefulness in reproduction and management of infertility is not well exploited; although vitamins E and C may protect spermatozoa against endogenous oxidative DNA and membrane damage.

As a result of active research in the area of evaluation of human semen, a series of sperm function assays have been developed. However, no single test is capable of evaluating all of the steps involved in fertilization. At present only a combination of assays complementing each other can provide a comprehensive evaluation of sperm function (Bar-Chama and Lamb, 1994; Sikka, 1996). Although, ideal tests of sperm function will markedly improve the clinician's ability to diagnose male factor infertility and help in its management, evaluation of the potential causes of sperm damage leading to abnormal sperm function and infertility is an important area of investigation.

It was therefore considered essential to determine the extent of antioxidant efficacy of some Nigerian medicinal plants aphrodisiacs towards lipid peroxidation in raw and cooked fish homogenate since mammalian spermatozoa membranes are rich in PUFA as an index of activity for accessing traditional aphrodisiac herbs.

## MATERIALS AND METHODS

### Interviews with Traditional Herb Sellers and Collection of Samples

A total of seventeen herb sellers (ages between 38 to 62 years) were interviewed in Mushin-Olosha open herbal market, in Lagos Nigeria using unstructured questionnaires (February to May 2006). Out of these, 15 were women and 2 men.

The herb sellers that consented were asked to give their knowledge on herbs that have been used successfully to boost libido, induce erection, increase sperm count and male fertility. The ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through interviews and discussions using a more qualitative conversational technique in their local language. Discussions allowed descriptive responses on the plant prescribed, such as part of the plant used, detailed information about mode of preparation (i.e., decoction, paste, powder and juice), form of usage either fresh or dried and mixtures of other plants used as ingredients. The conversations were built on trust with the common goal to improve the health situation in the country and to preserve and increase the knowledge on medicinal plants. We bought the medicinal specimens in order to gain their confidence and to cooperate economically with them as earlier reported by Marcia *et al*. (2005) and used by Sofidiya *et al*. (2007). The plants were identified at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos through literature (Gbile, 1984; Keay and Onochie, 1964a, b) using local names. Further confirmation of identity was carried out with assistance of Mr. Wale Ekundayo at the Forestry Research Institute of Nigeria (FRIN) herbarium Ibadan. Museum specimens are kept in our Laboratory.

### Fidelity Index

An index fidelity level (FI) was determined among the herb sellers according to the following formula.

$$FI\ (\%) = (N_H / N_T) \times 100\%$$

Where:
$N_H$ = No. of herb sellers that mentioned a particular plant
$N_T$ = No. of times a particular plant was mentioned

### Extraction Procedure

The root plants were washed with sponge and water to remove extraneous materials and other contaminants. They were dried in the open air, but under a shade (each spread on the shelf with used newspaper material) for 7 days. Some active constituents in plants can be denatured by direct exposure to sunlight. Each root plant was blended into powder to increase the surface area for reaction. Fifty grams of the powdered specimen of plant materials were each extracted in a one litre, transparent, air-tight glass container with 500 mL of 50% ethanol using cold extraction method for five days. The mixture was vigorously shaken everyday and left to stand on the shelf. The coloured solution obtained from each of the plant material was concentrated on the water bath. The concentrate (extract) obtained was put in a transparent plastic specimen bottle, labelled and preserved in the refrigerator at 4°C for further use.

### Fish Processing

Fresh but frozen Titus fish (*Scomber japonicum*) were obtained from the local market, washed and fleshy muscular part was used.

**Raw Fish Tissue Homogenate**

Twenty gram of raw fish muscle tissue was turned into a paste in a mortar. Two hundred milliliter of distilled water was added and the mixture blended thoroughly in an electric blender (10%W/V). The mixture was poured into a conical flask and 10 mM sodium phosphate buffer containing 0.15 M sodium chloride, at pH of 7.2 was added. The mixture was filtered and unbroken cells and cell debris were removed by centrifugation at 1500r for 15 min. The supernatant thus obtained was termed as homogenate and used for the *in vitro* lipid peroxidation study. Lipid peroxidation studies were performed almost immediately after the homogenate preparation.

**Cooked Fish Tissue Homogenate**

The method is the same as in raw fish tissue homogenate preparation, but the fish muscle was cooked at a temperature of 100°C for 15 min before blending.

**Lipid Peroxidation Method**

1 mL of each extract at the different concentrations were added to 1 mL of the homogenate and mixed together. Thiobarbituric acid (TBA) reactivity in the homogenate was determined by following a modified method of Luotola and Luotola, 1985. To 2 mL of each of the mixed homogenate was added 3 mL of 20% trichloroacetic acid (TCA) and mixed and centrifuged for 15 min. To 2 mL of supernatant, 1 mL of 2-Thiobarbituric acid (0.67%W/V) was added, mixed and kept in boiling water bath for 10 min, cooled to room temperature. The TBA chromogen (intensity of the pink coloured complex) was measured at 532 nm against blanks using UV Spectrophotometer, Thermo spectronic (Genesys 20 model). Water soluble vitamin E was used as reference standard. A graph of absorbance against concentration was plotted using the data obtained for the pure vitamin E. The TBARS of the extracts were evaluated from the standard curve (absorption against concentration of tetraethoxypropane) and expressed as n mol TBARS per mg of tissue.

**Statistical Analysis**

All the data were presented as the mean±standard error of the mean.

## RESULTS AND DISCUSSION

Women dominate the herbal medicine market in Nigeria. In this study, 88.9% were women. Most of them have an approximate idea about when they were born, but not the exact year. Survey results are presented in Table 1. These herbs have been used successfully to boost libido, induce erection, increase sperm count and consequently male fertility. All were root drugs, attributed to the doctrine of signatures, the roots being the life of the plant will generate life. They are all used as poly herbal preparations by combining two or more of the herbs together to achieve maximum results and enhance male fertility. Fidelity level is applied in order to compare the level of confidence in data obtained during survey (Togola *et al*., 2005). The calculated fidelity level index (Table 1) for the most frequently used was highest in *A. djalonensis* and lowest in *D. cayenensis*. It is interesting to note that only *Cissus polpunea* recorded scientific evidence in literature on its use as a traditional aphrodisiac. 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 Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) were elevated by 11 and 29% respectively (Smith *et al*., 2002).

The effect of extracts on lipid peroxidation in raw and cooked fish homogenate was measured as the amount of TBARS (nmol $mg^{-1}$ tissue). Lipid peroxidation was estimated following the formation

Table 1: List of traditional aphrodisiac herbs used in south west Nigeria

| Botanical name and family | Local name | Part used | Fidelity index (%) |
|---|---|---|---|
| *Anthocleista djalonensis* A. Chev. (Loganiaceae) | Hausa: Kwarii<br>Igbo: Okpokolo<br>Yoruba: Egbo sapoo | Root | 88 |
| *A. kerstingii* Gilg. | | | |
| *Carpolobia alba* G Don(Polygalaceae) | Igbo-Agba, Angalagala, | Root | 57 |
| *C. lutea* G Don(Polygalaceae) | Oziza, Uziza, Angalangala<br>Yoruba-Egbo orere,<br>Egbo oshunshun | | |
| *Cassia sieberiana* DC(Leguminosae) | Hausa: Araho, Gama fada,<br>Gamdafada, Gwazkiya, Margaa<br>Yoruba: Aidan toro, Aridan toro | Root | 58 |
| *Chasmanthera dependens* Hochst. (Menispermaceae) | Yoruba: Egbo atoh | Root | 72 |
| *Cissus populnea* Guill and Perr. (Vitacea) | Hausa-Daafaaraa, Malaiduwa, Maleduwa<br>Yoruba-Ogbolo pupa, Ogbolo | Root | 75 |
| *Cnestis ferruginea* DC(Connaraceae) | Hausa-Fura amarya<br>Igbo-Okpu<br>Yoruba-Egbo gboyingboyin | Root | 64 |
| *Dioscorea cayenensis* Lam.(Dioscoreaceae) | Hausa: Dooyar kudu<br>Igbo: Ji-Ogbagada, Ji-Oku, Ji-Oko, Ji-Okoocha, Yoruba: Egbo Igangan | Root | 23 |
| *Lecaniodiscus cupanioides* Planch (Sapindaceae) | Hausa: Kaa-fi-naamaa-zaaki<br>Igbo: Okpu<br>Yoruba: Aka, Aka-isin, Akika | Root | 68 |
| *Microdermis keayana* J. Leonard (Pandaceae) | Igbo: Akpalataa<br>Yoruba: Idiakpata | Root | 54 |

of malonyldialdehyde (MDA)-like substances, which react with TBA thus forming a pink-coloured complex at 532 nm. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products (PUFA in fish oils). Reaction of MDA with TBA or its diethyl derivative has been applied widely to assess lipid peroxidation in biological material (Badcock *et al*., 1997).

The 9 plant extracts inhibited lipid peroxidation in raw and cooked fish homogenate, but the extent was significant in cooked fish homogenate. Iron salts present in fish homogenate are known to decompose lipids to generate peroxyl and alkoxyl radicals, both can abstract hydrogen (H) and propagate lipid peroxidation (D'Souza *et al*., 2006).

Cooking helps the fish fat to get into the medium and solubilise. The plants have better antioxidant or lipid inhibition in the cooked fish homogenate because cooking also alters the physicochemical nature of the membranes thereby, the solubility and site of action is favoured by having more access to the radical and thus better activity.

*A. djalonensis* was most active while *D. cayenensis* was least active in both raw and cooked fish homogenates. TBARS activity/Concentration ($R^2 = 0.9533$ for raw and $R^2 = 0.9739$ for cooked) (Table 2).

The experiment indicates that A. djalonensis is more potent than vitamin E as an antioxidant in the inhibition of lipid peroxidation in raw and cooked fish homogenates. Antioxidant activity of the nine plant extracts and vitamin E can mainly be attributed to the phenolic group. The phenolic group provides a labile hydrogen atom for abstraction by free radical like peroxyl or alkoxyl radicals and gets converted to phenoxyl radical. The antioxidant potency depends on the stability and reactivity of this phenoxyl radical. All the nine plant extracts contain cardiac glycosides and saponin and have antioxidant properties. They considerably inhibited lipid peroxidation in raw and cooked fish homogenates compared to vitamin E, hence effectively inhibits the free radical damage to bio molecules.

In conclusion, results of this study have provided evidence to support the acclaimed role of these nine medicinal plants as aphrodisiacs in traditional medicine. The aqueous extract of the roots of these

Table 2: Comparison on effect of extracts on lipid peroxidation in raw versus cooked fish homogenates

| | Raw fish homogenate (%) | | | |
|---|---|---|---|---|
| Samples | 5 | 10 | 20 | 30 |
| *Anthocleista djalonensis* | 4.7242±0.0128 | 5.1779±0.0032 | 5.7945±0.0257 | 20.9930±0.0064 |
| *Carpolobia alba* | 2.3633±0.1031 | 4.0196±0.0352 | 9.5385±0.0947 | 17.2137±0.6250 |
| *Cassia sieberiana* | 2.1893±0.0578 | 2.1004±0.6792 | 4.2891±0.0704 | 13.2993±0.6831 |
| *Chasmantera dependens* | 2.6633±0.0085 | 2.3467±0.0224 | 2.7067±0.0071 | 11.1217±0.0032 |
| *Cissus populnea* | 1.9023±0.0306 | 2.2745±0.0402 | 2.9059±0.0758 | 6.4129±0.0128 |
| *Cnestis ferruginea* | 2.7929±0.0475 | 2.9726±0.1949 | 4.1122±0.0456 | 10.6570±0.0945 |
| *Dioscorea cayenensis* | 1.6786±0.0921 | 1.5394±0.0140 | 3.1670±0.0557 | 19.1580±2.9429 |
| *Lecaniodiscus cupanioides* | 2.2874±0.0752 | 2.8466±0.5206 | 2.7985±0.0347 | 6.4611±0.0160 |
| *Microdermis keayana* | 2.6744±0.3191 | 2.4282±0.5806 | 2.0152±0.0000 | 4.6002±0.0128 |
| | Cooked fish homogenate (%) | | | |
| Samples | 5 | 10 | 20 | 30 |
| *Anthocleista djalonensis* | 6.3517±0.0147 | 7.5196±0.0000 | 10.8347±0.0212 | 27.3218±0.0000 |
| *Carpolobia alba* | 2.1504±0.0127 | 3.7565±0.0113 | 9.1257±0.0258 | 21.8497±0.0074 |
| *Cassia sieberiana* | 2.5201±0.0042 | 2.0744±0.0046 | 7.3458±0.0225 | 27.2409±0.0628 |
| *Chasmantera dependens* | 2.5911±0.0073 | 3.9646±0.0000 | 7.0621±0.5752 | 27.3021±0.0042 |
| *Cissus populnea* | 5.2696±0.0112 | 6.5207±0.0074 | 8.9151±0.0127 | 13.0724±0.0074 |
| *Cnestis ferruginea* | 2.3659±0.0236 | 2.8616±0.0106 | 4.5718±0.0085 | 20.2607±0.0043 |
| *Dioscorea cayenensis* | 1.9300±0.0073 | 1.8199±0.0000 | 4.1727±0.0042 | 27.3217±0.0000 |
| *Lecaniodiscus cupanioides* | 2.0255±0.0127 | 1.8149±0.0085 | 3.0587±0.0043 | 27.3242±0.0085 |
| *Microdermis keayana* | 1.7758±0.0074 | 2.1749±0.0112 | 3.7639±0.0042 | 13.7727±0.0112 |

*TBARS (nmol $mg^{-1}$ protein), * = (Mean of 3 readings±SEM)

plants may be adduced to increase the testosterone level of the blood, which may be due to its saponin component. The aqueous extract of the roots of these herbs may thus be used to modify impaired sexual functions, especially in men.

Women who are perimenopausal, menopausal, or post-menopausal-along with women who use oral contraceptives-can experience genital oxidative stress, which can result in vaginal dryness and a lack of sexual desire. Some of these herbs are also recommended for use by women to prevent internally generated heat that affects sperm motility according to the survey report gathered. The plants have been shown to have antioxidant activity so could also preserve the nitric oxide needed to dilate blood vessels in genital tissues, which in turn causes sexual arousal. They could also be delivered in the form of a lubricant to sensitive genital skin, energise nerve endings and improve sexual pleasure and intimacy. Hence, the application of ROS scavengers is likely to improve sperm motility and function. Thus lipid peroxidation could serve as a rapid assay for accessing traditional aphrodisiac herbs.

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