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Improved Fertility Potential and Antimicrobial Activities of Sesame Leaves Phytochemicals

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1. Introduction

Broadly speaking, reproductive infertility is defined as the involuntary absence of conception in a couple after a general period of at least one year of regular unprotected sexual intercourse (Shittu et al., 2005; WHO, 1991).

The rapid advancement in the field of human reproductive biology (*in-vitro* fertilization-IVF etc) in this era has attracted a lot of interest amongst scientists such as andrologists, gynaecologists, biologists and epidemiologists worldwide (Schwartz, 1980; Shittu et al., 2005; Shittu et al., 2006a).

In view of the fact that any methodology that is empirical in infertility study ultimately determine the overall result quality with lots of consequence on the potential treatment options and other prevention programmes available (Thonneau & Spira, 1990), makes it necessary for clarification of some confusing terms often used in infertility studies amongst the readers. Such that a couple is said to be a fertile if they have conceived in the past and infertile if they have not conceived after at least one year of unprotected sexual intercourse as the case may be. While, a fecund couple is one who can conceive and an infecund couple is one that is impossible to conceive for whatever reason (Leridon, 1977; Thonneau & Spira, 1990).

In addition, primary infertility is defined as the absence of conception and secondary infertility is seen as the inability of obtaining any further (second or other) conception (WHO, 1991). While, the incidence of infertility also defined as the number of new infertile couples who started consulting within a given period of time (usually 1 year) and in a given place in proportion to the total number of non-infertile couples in the same place at the beginning of the same period of time.

Infertility is a medical condition considered to be a public health problem based on its global impact on the socio-economic status and the quality of health life of the society at large and with a prevalence of over 80 million couples affected worldwide (Shittu et al., 2005; Shittu et al., 2006a; WHO, 1991). Such that approximately 15% of couples suffered from infertility worldwide (Nishimune & Tanaka, 2006; Norris, 2001) as compared to about 8% and 8.5% of couples affected in the US (Leke et al., 1993) and Canada (Norris, 2001) respectively.

Worldwide, infertility is more prevalence in the women than men with a traceable male factor accounting for 40–50% of infertile couples (De Kretser & Baker, 1999; Shittu et al., 2005; Shittu et al., 2006a; Nishimune & Tanaka, 2006; WHO, 1987). However, in developing nations of the world like Africa for example, where there are more infertile cases with limited or no treatment options especially assisted reproductive technology available for the management of this medical condition (Shittu et al., 2005, Shittu et al., 2006a; WHO, 1987), there is a higher prevalence rate of about 30–40% (Leke et al., 1993; Shittu et al., 2005, Shittu et al., 2006a) with male factor accounting for 30% of cases seen (Leke et al., 1993). Other previous studies have also shown that the infertility risk for older women aged 35–44 years appeared to be twice as high as that of young woman of 30–34 years and that the infertility risk for a black woman is also 1.5-times higher than the whites (Thonneau & Spira, 1990).

Hence, male fertility and subfertility have also attracted a lot of considerable interest amongst basic medical scientists and clinicians alike over the past few years now (Skakkebaek, 2003; Shittu, 2006, 2010; Shittu et al., 2005, Shittu et al., 2006a).

In addition, there is a rising prevalence of male factor infertility worldwide, as a result of exposure to environmental toxicants/endocrine disruptors and the infiltration of biogenetically engineered western diets and lifestyles among other factors into the developing worlds especially (Izegbu et al., 2005; Shittu, 2006, 2010; Shittu et al., 2007a, Shittu et al., 2008a). Such that more alarming is the decreasing sperm quality of human and wildlife species due to environmental exposures to these endocrine disruptor pollutants and chemicals that are believed to cause gene mutations as expressed over the past few years now (Duty et al., 2003, Sharpe & Irvine, 2004; Sharpe & Shakkebeak, 1993; Shittu, 2006, 2010; Shittu et al., 2008a; Vos et al., 2000; Zuping et al., 2006).

The major risk factors responsible for the increase in prevalence of infertility in the 20–24 year age group of women include STDs (sexually transmitted diseases) and their tubal consequences (Mosher & Aral, 1985; WHO, 1991).

The fact that microbial infections/STD (such as gonorrhea, syphilis, Chlamydia, Candidiasis) is one of the leading causes of infertility in both male and females couples especially in this part of the world (WHO, 1987; WHO, 1991) and with the associated problems of numerous unwanted effects of the synthetic chemical/hormonal agents (antibiotics and steroids etc) available as treatment options have both raised cause for concern in recent times (Shittu, 2006, 2010; Shittu et al., 2009).

Incidentally, the last decade have witnessed increasing intensive study on isolates and other active phytochemicals been extracted from plant species used in folkloric medicine for over thousands of years now (Bankole et al., 2007; Hanilyn, 1998; Nascimento et al., 2000; Shittu, 2010; Shittu et al., 2006b; Shittu et al., 2007b). Moreover, these agents are equally recognized by the World Health organization to have proven potential of treating microbial infections, diabetes, cancers and infertility/ subfertility among other known chronic medical conditions (Bankole et al., 2007; Shittu, 2010; Shittu et al., 2006b; Shittu et al., 2007a; Shittu et al., 2008a; Shittu et al., 2009).

In addition, in this era of ours, natural resources have been used to produce various bioactive compounds such as alkaloids, ethanol, organic acids, immunomodulator, vitamins

and polysaccharides etc that are of medicinal values in food, medical, chemical and biochemical industries (Hanilyn, 1998; Shittu, 2006, 2010).

Hence, expressed worldwide is the search for an ideal folkloric phyto-chemical medicinal agent with a broad spectrum and proven potential of treating infertility/ subfertility conditions including microbial infection among the other causes of infertility with minimal or no side effects as compared to their synthetic counterparts (Bankole et al, 2007; Shittu, 2006, 2010; Shittu et al, 2006b; Shittu et al, 2007, 2007b, 2007c; Shittu et al, 2008a, Shittu et al, 2009; Shittu et al, 2010).

The phytoestrogens appeared to be one of such natural estrogenic agents that have attracted so much attention in the last decade in view of their reported health benefits and they include four broad classes of phytochemicals namely the lignans (sesame seed and flaxseed), isoflavonoids (soybeans), stilbenes and coumestrol (Adlercreutz *et al.*, 1986; Murkies, 1998; Shittu, 2006, 2010). These agents mimic endogenous estrogens and depending on their concentrations, they either act agonistically or antagonistically by displaying the endogenous estrogens from the binding sites on the estrogens receptors (α and β) among its other mechanisms of actions (Kuiper et al, 1997; Shittu, 2006, 2010).

Based on the fact that they are consumed in large amounts in the diet, the metabolic effects noticed are usually that of antiestrogenic, thus, compete with the much more potent endogenous estradiol for the estrogens receptor (ER1 & ER2) binding sites and ultimately blocked their estrogenic activity (Martin *et al.*, 1978; Whitten & Naftolin, 1991).

In addition, sesame plant is one of the richest food sources of phytoestrogenic lignans, a valuable phytochemical known to man since the dawn of civilization (Thompson et al, 1991) and is now increasingly being incorporated into human diet worldwide because of their reported health benefits (Shittu, 2006, 2010).

The plant is rich in trace elements/minerals such as calcium, iron, magnesium, zinc, copper and phosphorus (Obiajunwa et al, 2005; Shittu, 2006, 2010; Shittu et al., 2006b, Shittu et al, 2009).

All parts of the sesame plant such as the seed, oil and leaves are also useful and are locally consumed as a staple food by subsistence farmers in the Northern, South-west and Middle - belt regions of Nigeria and celebrated also in folkloric medicine in Asia and Africa (Akpan - Iwo et al, 2006; Shittu et al, 2006b; Shittu, 2010). Thus, this may account for the high fecundity among the adult male population in these particular areas (Shittu, 2006; Shittu, 2010). The local names of the plants depend on the source areas of cultivations in the world, such as ekuku-gogoro (Yoruba- *Sesamum radiatum*), yanmoti (Yoruba-*S. indicum*), ridi (Hausa) and beni (Tiv/Idoma and English) or gingelly (English) (Gill, 1992; Shittu, 2006, 2010; Shittu et al, 2009).

Recent study on bioavailability of sesame plants consumption in humans have shown that the lignans usually undergo extensive metabolism in the intestine depending on the characteristics of the individual intestinal microflora to produce mammalian lignans-enterolactone especially (Penalvo et al., 2005; Shittu et al, 2009). Moreover, plasma lignans concentrations showed a linear correlation with urinary excretion of lignans (Penalvo et al., 2005; Shittu et al, 2009).

Once, spermatozoa are released from the testis, they enter into the epididymis, which is a steroid-dependent organ for storage and then undergo another physiological maturation

(capacitation) process involving other various morphological and biochemical changes with the resultant initiation of progressive motility and acquisition of fertilizing ability by the matured epididymal spermatozoa (Shittu, 2006, 2010; Shittu et al, 2007a).

The epididymal sperm cells are also susceptible to oxidative damage from reactive oxygen species due to their lack of proper cytoplasm and fragile membrane nature (Aitken & Fisher, 1994; Shittu, 2006, 2010; Shittu et al, 2006a; Shittu et al, 2007a). No doubt, the acquisition of sperm motility is seen as an integral part of the whole epididymal sperm maturation process (Orgebin-Crist, 1967), which occurs in the micro-environment provided by the epididymal secretory products and its antioxidant enzymes such as sialic acid, acetyl carnitine, glyceryl-phosphoryl choline (GPC) among others. These epididymal secretions helped to maintain the basic osmolarity of the epididymal luminal fluid (Wales et al., 1966), which is steroid regulated (Schwaab et al, 1998) and assist in stability of the spermatozoal membranes (Scott et al, 1963; Zini & Schlegel 1997; Shittu, 2006, 2010). All these activities are important in the metabolism of spermatozoa after capacitation (Mitra & Chowdhury, 1994; Shittu, 2006, 2010).

In addition, most of the testicular fluid (about 96%) is reabsorbed by the non-ciliated cells in the efferent ductules (Clulow et al., 1998) and without this fluid re-absorption, the sperm will remain over diluted and incapable of maturation within the epididymis and as such, any blockage or interference in normal functioning of the estrogen receptors or estrogens may result in infertility (Oliveira et al, 2000; Shittu, 2006, 2010)..

1.1 Aims and objective

As a result of paucity of knowledge and folkloric claim on the *Sesamum radiatum* leaves effectiveness in treating infertility and infections, our aims and objectives are to determine the following:

- i. The antimicrobial activity of the aqueous crude extract of *Sesamum radiatum* leaves phytochemicals (essential oils and phytoestrogenic lignans) on some selected microorganisms
- ii. The histomorphometric and stereological evidence of fertility potential of sesame leaves phytochemicals on adult male Sprague Dawley (SD) rats` epididymal tubules.

2. Material and methods

2.1 Collection of plant materials

Sesame radiatum plants (Schum and Thonn - Pedaliaceae family) were purchased from a vendor in Agege market, Lagos after being identified by Dr. Shittu in May 2011. However, the plant was initially authenticated by the herbarium section of Forestry Institute of Research (FRIN) with FHI # 107513 on the 5th of August, 2005 (Shittu, 2006; Shittu *et al.*, 2006). In addition, voucher specimens were deposited in Botany Departments of University of Ibadan and Lagos State University, respectively.

2.2 Preparation of aqueous crude extracts

The leaves having been separated from the rest of the plants were air dried for 2 weeks and later grounded into a powdery form using a grinder. 100g of the powdered leaves were

added to 1.0 litre of distilled water at a ratio of 1:10 in a clean dried beaker and allowed to boil to boiling temperature after intermittent stirring on a hotplate for one hour. The decoction was filtered into another clean beaker using a white sieve clothing material and the filtrate evaporated at 50 °C to dryness in a desiccator to produce a black shining crystal residue form with a yield of 83% w/w of the extract. The crude extract was kept in the refrigerator (4°C) before being reconstituted and later used for the in-vivo study.

2.2.1 Preparation of aqueous, ethanolic and methanolic extraction of sesame leaves

Aqueous, ethanolic and methanolic extraction of Sesame leaves using modified Okogun, (2000) method of extraction was adopted in the process (Shittu et al, 2007c) for the present antimicrobial studies. Such that the diluents used were absolute methanol, ethanol and sterile distilled water respectively. 1g of the raw air-dried and grinded Sesame leaves was added to 10ml of each diluent in a 20ml screw cap bottle. The modification was in the extraction time, which was for 5 days (120 hours) and the storage of the solution took place in the refrigerator at 4°C. The extracts obtained were regarded as the full concentration. Methanolic, ethanolic and aqueous extracts of *Sesamum radiatum* leaves were studied for their *in-vitro* antimicrobial activity against both gram positive and gram negative microorganisms and yeast using Agar diffusion method.

2.3 Phytochemical screening using gas chromatography-mass spectral

Crude methanolic extracts of Sesame leaves were analyzed by GC/MS. GC analyses were performed using a Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230°C respectively. A fused silica capillary column HP-InnoWax (30 in x 0.25 mm, film thickness 0.25 (μm)) was used. The oven temperature was held at 50 °C for 5 min holding time and the temperature was raised, from 50-230°C at a rate of 2 °C /min. Helium was the carrier gas at a flow rate of 22cm/sec. One millilitre of extract mixed with methanol (80%), at a split ratio of 1:30 was injected (Shittu et al, 2006b; Shittu et al, 2007b).

GC/MS analyses were carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890) equipped with NBS 75K Library Software data. The capillary column and GC conditions were as described above. Helium was the carrier gas, with a flow rate of 22cm/s. Mass spectra were recorded at 70 eV/200°C. The scanning rate of 1scan/sec and the run time was 90 minutes as described in our previous study (Shittu et al., 2007b).

Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices (RI) and by comparison to reference compound (Shittu et al., 2007b).

2.4 Selection of micro-organisms

Staphylococcus aureus (clinical), *Streptococcus pneumonia* (clinical) and *Candida albicans* (clinical) were the microorganisms used for this study and they were obtained from the Microbiology Laboratory of the Lagos State University Teaching Hospital (LASUTH). These microorganisms were identified and confirmed at the Microbiology department of the Drug

Quality Control Laboratory, LASUTH, Ikeja, Lagos. In addition, standard strain of *Staphylococcus aureus* (ATCC 29213) of oxoid Culti-loop (Oxoid Ltd., Hampshire, England) was also used. The choice for the selection of these organisms was based on their high resistance to common available antibiotics and their implication in pelvic inflammatory disease, one of the leading causes of infertility.

2.4.1 Preparation of 24 hours pure culture

A loop full of each of the selected *microorganisms* was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37°C for 24 hours except for *Candida albicans* that was incubated at 25°C for 24-48 hours.

2.4.2 Standardization of micro-organisms

Each of the 24 hour old pure cultures was suspended in a Roux bottle containing 5ml of physiological saline. Each suspension of *microorganisms* was standardized to 25% transmittance at 560nm using an Ultraviolet (UV) -visible spectrophotometer.

2.5 Standard antibiotics used for study comparison

The primary standard of Cloxacillin obtained from Sigma-Aldrich (St Louis, MO, USA) was used for this study. However, the secondary standards with antibacterial and antifungal activity used for comparison with the various crude extract of sesame were obtained from local manufacturers in the Nigeria.

2.5.1 Antimicrobial screening

The modified Collin et al (1995) agar-well diffusion method was employed to determine the antimicrobial activities for the various crude extracts. Various concentrations of each of the aqueous, ethanolic and methanolic extracts respectively was made by diluting 1ml of each reconstituted extract in 2ml, 4ml, 6ml and 8ml of sterile distilled water respectively.

2.5.2 The Mean Inhibitory Concentration (MIC)

The MIC of the various crude extracts of sesame leaves against the tested *micro-organisms* was obtained. Agar-well diffusion method using Modified Collins et al (1995) was employed; approximately 10ml of sterile Muller-Hinton Agar (MHA) was poured into sterile culture plates and allowed to set. About 10ml of the antibiotic medium No 2 seeded with 0.5ml of 24 hours old culture of bacteria isolates was layered onto the MHA and allowed to set. The seed medium was then allowed to dry at room temperature for about 30 minutes. In the case of *Candida albicans*, Sabouraud Dextrose Agar (SDA) seeded with a 24 hours old *Candida albicans* was layered on the MHA. With the aid of a sterile cork borer, wells of about 8mm in diameter were punched on the plates. About 0.5ml of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37°C for 24 hours except for the plates seeded with *Candida albicans*, which were already incubated at 25°C for 24-48 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

2.5.3 Measurement of Zone of Inhibition (ZI)

The zones of inhibition of the tested *microorganisms* by the various crude sesame extracts were measured using a Fisher-Lilly antibiotic zone reader model 290 (USA). The diameter sizes in mm of the zone of inhibition are shown in the respective tables below.

2.5.4 Minimum Inhibitory Concentration (MIC) of each extract

The MIC for each selected *microorganism* used was determined using microdilution method previously described (Bankole et al, 2007; Eloff, 1998; Shittu et al, 2006b; Shittu et al, 2007b)) as the last dilution of the extract that inhibited the growth of the tested pathogenic *microorganisms*. The various MIC are shown in the respective tables below.

2.6 Animal

Thirty matured and healthy adult male Sprague Dawley rats weighing 120 to 216g were procured from Ladoke Akintola University, College of Medicine, Ogbomosho and housed in a well ventilated wire-wooden cages in the departmental animal house. They were maintained under controlled light schedule (12 hours Light: 12 hours Dark) at room temperature (28°C) and with constant humidity (40-50%). The animals were allowed to acclimatize for a period of 7 days before treatment during the experiments. During this period they were fed with standard rat chows/pellets supplied by Pfizer Nigeria Ltd and water *ad-libitum*. Individual identification of the animal was made by ear tags (Shittu et al, 2007a, Shittu et al, 2008, Shittu, 2010).

2.6.1 Experimental procedure

The rats were randomly divided into three groups (A to C) comprising of ten rats each. The group A served as the control while B and C constituted the treated groups.

The animals in group A received equal volume of 0.9% (w/v) normal saline daily while group B received aqueous extract of sesame leaves at 14.0mg/kg body weight /day.

The animals in group C were given aqueous extract of sesame at 28.0mg/kg body weight /day (twice the group B dose). All the doses were given via gastric gavage (oro-gastric intubation) daily for a period of 6 weeks. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals.

All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, lethargy, refusal of feeds, weight loss, hair-loss, coma and death) throughout the duration of the experiment.

2.6.2 Animal sacrifice

The rats were anaesthetized at the time of sacrifice by being placed in a sealed cotton wool soaked chloroform inhalation jar between 0900 and 1100 hours done the following day after

the termination of the experiment after post over night fasting of the animals. The weights of the animals were taken weekly and before the sacrifice (Shittu et al, 2007a, Shittu et al, 2008, Shittu, 2010).

2.7 Organ harvest and tissue processing for light microscopy

The Epididymes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. Their weights were noted and volume measured by water displacement with the aid of a 10ml measuring cylinder. Later, the sizes (length and width) were recorded by use of a sliding gauge ($d = 0.1$) before being fixed in freshly prepared 10% formol saline solution. The fixed tissues were transferred into graded alcohol and then processed for 17.5 hours in an automated Shandon processor after which were passed through a mixture of equal concentration of xylene. Following clearance in xylene the sections were then infiltrated and embedded in molten paraffin wax. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axes of the epididymes. These sections were designated “vertical sections”. Serial sections of $5\mu\text{m}$ thickness were cut, floated onto clean slides coated with Mayer’s egg albumin for proper cementing of the sections to the slides and were then stained with Haematoxylin and eosin stains. (Adelman and Cahill, 1989; Shittu, 2006, 2010; Shittu et al, 2007a).

2.8 Determination of morphometric parameters

Epididymal volume and weight estimations were done as stated above. Parameters of the two epididymes from each rat were measured and the average value obtained was regarded as one observation. However, for each epididymis, seven vertical sections from the polar and the equatorial regions were taken by systematic random sample method to ensure fair distribution between the polar and equatorial regions of each epididymis (Shittu, 2006, 2010).

The following morphometric parameters namely the volume and weight; diameter and cross-sectional area of the epididymal tubules were determined.

The diameter (D) of epididymal tubules with profiles that were round or nearly round for each animal was estimated. A mean diameter “D”, was taken as the average of two diameters, D1 and D2 (where D1 is the short axis while D2 is the long axis; both D1 and D2 are perpendicular to each other). D1 and D2 were considered only when the ratio of D1:D2 or $D1/D2 > 0.85$ (Form factor) (Shittu, 2006, 2010; Shittu et al., 2006b; Shittu et al, 2009).

The cross-sectional areas (AC) of the epididymal tubules were determined from the formula $AC = \Pi D^2/4$, (where Π is equivalent to 3.142 and D = the mean diameter of the epididymal tubules (Shittu, 2006, 2010; Shittu et al., 2006b; Shittu et al, 2009).

2.8.1 Determination of stereological parameters

Serial transverse sections of $5\mu\text{m}$ of the H and E-stained specimens prepared were subjected to un-biased stereological techniques modified from previous report (Mouton, 2002; Shittu et al., 2006, 2010; Shittu et al, 2006c). Each image of the epididymal tubules at a magnification of 400 X was projected and drawn on a 16-point grid, completely counted in six different fields to make a total of 96 point-test grid for each of the systemic randomly selected section such that 5 sections/rat were taken from each group. Manual point intercept

counting methods consist of a counting grid made up of a series of crosses in a regular and uniform square array. The density of crosses was such that one cross represented an area of 4 cm² on the counting grid. The total number of crosses (circled or otherwise) falling on each structure per each section of specimen was counted and then added-up to get the final estimated result. Using this procedure, the volume density of the stroma, epithelia lining and tubular lumen of epididymal tubules were estimated as previously described (Weibel & Gomez, 1962; Shittu, 2006, 2010; Shittu et al, 2008b; 2009). The percentage volume density was determined by multiplying the volume density by 100.

2.9 Statistical analysis

The weight data were expressed in Mean \pm S.D (Standard deviation) while other data were expressed as Mean \pm S.E.M (standard error of mean). Comparisons between groups were done using the student's *t*-test, ANOVA and non-parametric Mann-Whitney U test with input into SPSS 12 software Microsoft computer (SPSS, Chicago, Illinois). Statistical significance was considered at $P \leq 0.05$ as used in previous studies (Shittu, 2006, 2010; Shittu et al, 2007a; Shittu et al, 2008).

3. Result

No obvious signs of toxicity such as weakness, lethargy, tremors, refusal of feeds, weight loss, hair-loss, coma and death were seen in any of the animals. Moreover, most of the animals exhibited calmness; improve appetite for food and water and general sense of well-being, all through the duration of the study as reflected in the increase in their body weight through the study duration.

The GC-MS showed that the methanolic extract of *sesamum radiatum* leaves contained mainly essential oils such as aromatic phenolic compounds- sesamol, sesaminol, sesamin, phosphorus, calcium, vitamin C, carboxylic acids and other classes of compounds including fatty acids like palmitic acids, arachidonic/arachidic acid, stearic acid, myristic acid, oleic acid, linoleic acids, thiazole, pyrroles, disulphide and aldehyde. In addition, it confirmed the presence of trace minerals, vitamins and steroids such as adrostenedione among others in the leaves of *sesamum radiatum* plant.

Combination of both methanolic and ethanolic extracts of *sesamum radiatum* leaves would have a broad spectrum antimicrobial effect against all the tested clinical micro-organisms except for the aqueous extract that has mild inhibitory activities on *Candida albican* only as shown in table 1.

The results obtained in table 1 showed that the methanolic extract demonstrated a mild antimicrobial activity against *Staphylococcus aureus* at both the full concentration and 1:2 dilution of the extract without any inhibitory effect on either the tested *Streptococcus pneumoniae* or *Candida albicans*. While, the ethanolic extract had a very strong antimicrobial effect against *Streptococcus pneumoniae* at full concentration including a strong and mild antimicrobial effect on *Candida albicans* at both full and 1:2 dilution of the extracts respectively. However, ethanolic extract has no inhibitory effects on tested *Staphylococcus aureus*. *Candida albicans* growth on the culture plates was also mildly inhibited by the aqueous extract at full concentration, 1:2; 1:4 and 1:6 dilutions of the extracts and with no other antimicrobial effects observed against the tested *Streptococcus pneumoniae* and *Staphylococcus aureus*.

Microorganisms	Crude Extract	Sensitivity				
		Full	1:2	1:4	1:6	1:8
<i>S. aureus</i>	Methanolic	+	+	-	-	-
	Ethanolic	-	-	-	-	-
	Aqueous	-	-	-	-	-
<i>S.pneumoniae</i>	Methanolic	-	-	-	-	-
	Ethanolic	+++	-	-	-	-
	Aqueous	-	-	-	-	-
<i>C. albicans</i>	Methanolic	-	-	-	-	-
	Ethanolic	++	+			
	Aqueous	+	+	+	+	-

(+) susceptibility (inhibition zone ≥ 10 mm)
(-) absence of susceptibility

Table 1. Showed the sensitivity of 3 tested micro-organisms to the different crude extracts of *Sesamum radiatum* leaves

Microorganisms	Crude Extract	Full	1:2	1.4	1:6	1: 8
	Methanolic		39.3			
	Ethanolic					
<i>S. aureus</i>	Aqueous					
	Methanolic					
	Ethanolic	76.2				
<i>S.pneumoniae</i>	Aqueous					
	Methanolic					
	Ethanolic		28.2			
<i>C. albicans</i>	Aqueous					31.0

The MICs is measured in µg/ml of the crude extracts on tested microorganisms

Table 2. Showed the minimum inhibitory concentrations (MICs) of different crude extracts of *Sesamum radiatum* leaves against the tested microorganisms.

Name of antibiotic/ antifungal	Zone diameter of tested Microorganisms			Concentration Used (µg/ml)
	<i>Staph. aureus</i>	<i>Strept pneumoniae</i>	<i>Candida albicans</i>	
Fluconazole tablet 500mg (2o standard)	-	-	40mm	100
Clotrimazole Vaginal tablet (2o standard)	-	-	35.5mm	100
Ampiclox tablet 500mg (2o standard)	30.5mm	-	-	100
Amoxycillin (2o standard)	15.5mm	11.5mm	-	100
Cloxacillin tablet(1o standard)	30 mm	-	-	100

Table 3. Antimicrobial effect of primary (1o) standard(Cloxacillin) and secondary (2o) standard antibiotics (Amoxycillin tablet, Ampiclox tablet, 500mg) and antifungal (Clotrimazole vaginal tablet 200mg and Fluconazole tablet, 500mg) on tested pathogenic microorganisms.

We observed a significant ($P < 0.05$) body weight gain using one-way ANOVA in all the treated animals. There was significant weight gain in both raw weight and relative weight of the epididymis per 100g body weight in a dose dependent manner as seen in Table 6.

Weight	A(Control)	B (High dose)	C (Low dose)
Initial (pre-experiment) (g)	127.3 \pm 5.55	206.2 \pm 6.45	186.3 \pm 1.99
Final (post- experiment) (g)	185.2 \pm 11.05*	248.2 \pm 14.40*	219.8 \pm 4.47*
weight gain (g)	58.5 \pm 5.50*	42.0 \pm 7.95*	33.4 \pm 2. 48*

Values are mean \pm S.D. *P < 0.05 was considered significant.

Table 4.a) Average weekly body weight of animal.

Group	Raw weight (g)	Epididymo-somatic weight (wt/100 g bwt)
Group A (control)	0.55 \pm 0.03	–
Group B (high dose)	0.75 \pm 0.01*	0.30 \pm 0.00*
Group C (low dose)	0.57 \pm 0.01*	0.26 \pm 0.01*

Values are mean \pm S.D.

*P < 0.05 was considered significant.

Table 4.b) Summary of weight (g) of epididymis.

The mean epididymal diameter and volume density of the tubular lumen significantly ($P < 0.05$) increased by 65% and 71% respectively in low dose sesame as compared to the control group. Similar findings in high dose sesame were also observed.

Group	N	Mean \pm S.E.M
A(control)	10	3.4 \pm 0.4
B (high dose)	10	1.6 \pm 0.26*
C (low dose)	10	0.8 \pm 02 *

The epididymal tubular profile of group C is the lowest of all the groups. Group B is 2.0 times higher than group C. This also correspond to the epididymal weight per 100g body weight of the animals
Values are mean \pm S.E.M.

*P < 0.05 was considered significant.

Table 5.a) Summary of epididymal tubular profile

Group	N	Mean \pm S.E.M
A	18	217.8 \pm 8.9
B	9	242.6 \pm 32.4*
C	4	358.4 \pm 21.0 *

Group C has the largest tubular diameter of all the groups.

Values are mean \pm S.E.M.

*P < 0.05 was considered significant.

Table 5.b) Summary of epididymal tubular diameter

Group	N	interstitium	surface epithelia	tubular lumen
A	10	4.6 + 0.4*	7.0 + 0.6*	.3.8 + 0.7*
B	10	4.0 + 0.5*	3.2 + 0.9*	8.0 + 0.7*
C	10	4.0 + 1.1*	4.2 + 1.0*	6.6 + 0.8*

Group B and C have the same significant interstitial activity; Group A has the highest epithelial activity. While, B is 1.3 times more than A in epithelial activity. Group B has the highest significant luminal activity with 1.3 times more than B group in its luminal activity.
Values are mean ± S.E.M.
*P < 0.05 was considered significant.

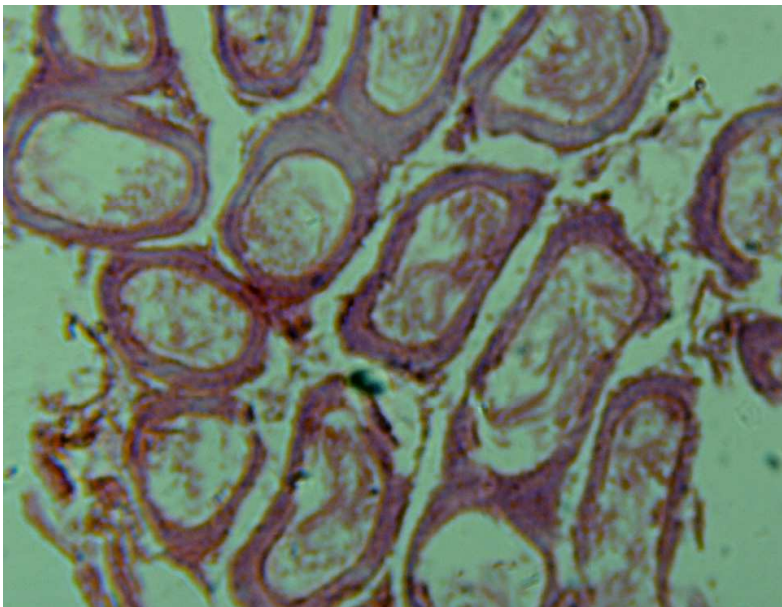
Table 5.c) Summary of volume fractions of Epididymal tissue profile

Group	N	Mean ± S.E.M
A	10	0.21 ± 0.02
B	10	0.1 ± 0.02*
C	10	0.06 ± 0.01 *

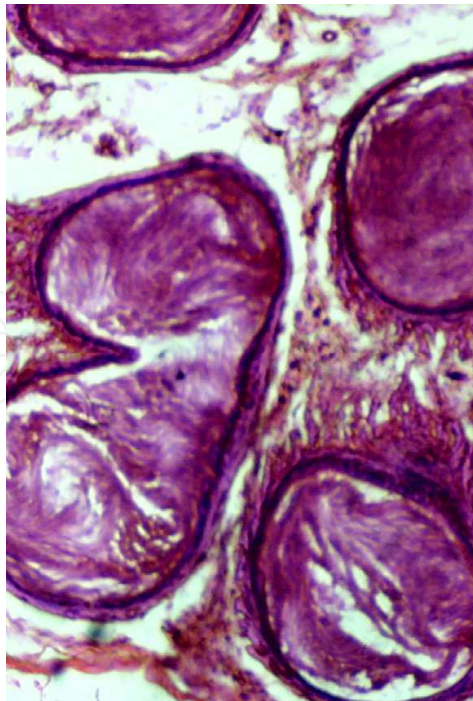
Group C has the lowest significant epididymal density of the groups; B group is twice C group in their density.
Values are mean ± S.E.M.
*P < 0.05 was considered significant.

Table 5.d) Summary of Numerical density (Nv) of epididymal tubular profile

Also, the high dose group showed evidence of matured spermatozoa fullness within the varying tubular lumens of the different sizes epididymal tubules seen in the photomicrographs fig 2 and 3.in the X100 magnifications. Hence, the epididymal lumen appeared wider and fuller with matured spermatozoa when compared to the control as seen in figure 1-3.

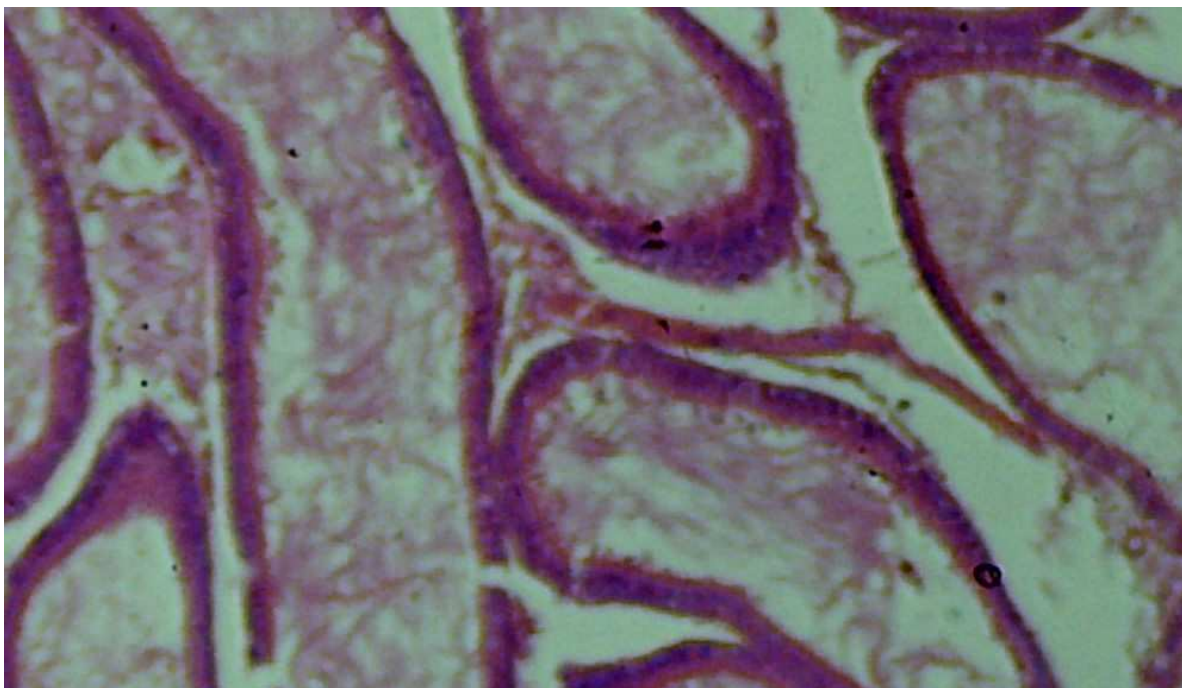


Group A micrograph showed normal sized and sparsely fullness of the epididymal tubules with spermatozoa.as compared to the treated animal groups. Group A X 100 magnification.
Fig. 1. Micrographs of the epididymes of the animals in group A



Group B micrograph showed widening and fullness of the epididymal tubules with matured spermatozoa. Group B X 100 magnification.

Fig. 2. Micrographs of the epididymes of the animals in group B



Group C micrograph showed widening and fullness of the epididymal tubules with matured spermatozoa. Group C X 100 magnification.

Fig. 3. Micrographs of the epididymes of the animals in group C

4. Discussion

There is increasing role and contribution of sesame lignans and other essential oils obtained from *Sesamum radiatum* research to medicine in this present decade.

Moreover, *Sesamum radiatum* leaves rich in trace minerals, vitamins, antioxidant lignans (phytoestrogens) and other essential oils as reflected in the GCMS findings, have the ability of improving fertility potential of the male reproductive tract with antimicrobial impact against the common infective agents of the reproductive tract.

Muller-Hinton Agar diffusion (MHA) method was extensively used to investigate the antibacterial activity of natural antimicrobial substance and plant extracts (Ahmed et al, 2010; Bankole et al, 2007; Shittu et al, 2006b). However, for solution/extracts with a low antimicrobial activity, one will need a large concentration or volume made possible with holes or cylinders using MHA rather than the disk method with limited applications (Bastner, 1994; Ahmed et al, 2010; Bankole et al, 2007; Shittu et al, 2006b).

The GC-MS of the methanolic *Sesame radiatum* leaves extract did show the presence of mainly aromatic phenolic essential oils, which possess antimicrobial properties (Alma et al, 2003). For example, sesamol as one of the most potent antioxidants in the leaves was also reported in our previous study (Shittu et al, 2007c).

Moreover, the methanolic extracts showed antibacterial effect against *Staphylococcus aureus* only at a higher concentration. Unlike, the ethanolic extracts with no inhibitory activity against *Staphylococcus aureus* and was very effective against *Streptococcus pneumoniae* and *Candida albicans*, Hence, possesses both antibacterial and antifungal activity (R'ios & Recio., 2005). This same natural antibacterial effect against common skin pathogens such as *Staphylococcus* and *Streptococcus* bacteria as well as common skin fungi including the athlete's foot fungus was reported in other similar study using the sesame oil (Sesame, 2000).

The pH of compounds in dilutions had also been found to modify the results outcome as usually observed in the case of phenolic or carboxylic compounds present in plants extracts, since studies have shown that the different effects of neutral essential oil are pH dependents. For example, anise oil has a higher antifungal activity at pH 4.8 than at 6.8, and *Cedrus deodorawas* oil was most active at pH 9.0 (Janssen et al 1976).

In the present study, ethanolic extracts with lesser acidity was found to be more effective against *Candida albicans* at a lower pH than the methanolic extracts with no inhibitory activity against *Candida albican* as shown in table 1.

Moreover, the aqueous extract showed antifungal activity at a higher pH as seen in table 1. However, the antifungal activity of ethanolic extract was more potent than that of the aqueous extracts as reflected with lower MICs against *Candida albicans* with value of 28.2 µg/ml when compared to 31.9 µg/ml. obtained for the aqueous extract as shown in table 2.

This in a way reflect on the significance of the preservation of some of the active ingredient present in the leaves like sesame lignans such as sesaminol and its glucosides, which are water soluble in nature and extracted effectively during extraction processes of the Sesame leaves (R'ios & Recio., 2005). Hence, ethanol was more effective than methanol in extraction and preservation of the oily and water soluble active ingredients with proven anti-microbial properties especially against yeasts (Shittu et al, 2007c).

This preservation was further enhanced and improved using the modified Okogun's method as supported by other previous report (Shittu et al, 2007c) and complementary to effective antimicrobial property of *sesamum radiatum* leaves (Shittu et al, 2006).

In addition, antimicrobial effectiveness of the different crude sesame leaves extracts were similar to that of standard antibiotics and antifungals used as reflected in the tables 4 and 5 respectively. More so, the zone of inhibition obtained especially against the *staphylococcus aureus* of the methanolic extract of sesame leaves (39.3 mm) was found to be higher than that of the primary standard antibiotic-cloxacillin (30.0mm) used in this study. This also implied a relative effectiveness of the *sesamum radiatum* leaves extract over the regular standard primary synthetic and expensive antibiotic available in the market.

It is actually the preservation of these rich nutritive constituents of aqueous *sesamum radiatum* leaves extracts that has contributed to the general state of well-being observed in all the treated animals during the whole experimental period with sesame-treated rats showing evidence of significant raw weight gained ($P < 0.05$). However, with ANOVA, no significant difference was observed in the raw animal weights compared to control.

Interestingly, other studies have reported the increasing application of stereological and morphometric techniques as new veritable approaches in modern medicine and biomedical sciences/researches in recent years (Mukerjee & Rajan, 2006, Shittu, 2006, 2010; Shittu et al, 2008b; Shittu et al, 2009).

In this present study, matured adult male rats were used based on the fact that morphometric study using light microscopy is best evaluated when the studied organ has attained a reasonably sizable dimension (Mukerjee & Rajan, 2006, Shittu, 2006, 2010; Shittu et al, 2008b; Shittu et al, 2009).

There was relative significant ($P < 0.05$) epididymal weight gain difference in the sesame-treated animal compared to the control as shown in table 6b. However, no significant changes in epididymal weights were observed in estradiol (high and low dose) treated golden hamsters (Jin *et al.*, 2005).

In the present study, it appeared that sesame-treated rats have more significant ($P < 0.05$) effects on the raw weights of the epididymis than testis as observed in other previous studies (Shittu, 2006, 2010; Shittu et al, 2008a; Shittu et al, 2009). In addition, this is actually a reflection of the more active site of action of sesame phytoestrogenic lignans as the epididymis is a known steroid responsive organ (Shittu, 2006, 2010). Hence, the activities of sesame is hormonally influenced as efferent ductile and epididymis of rats are also rich in estrogens receptors; a and b sub-types (Hess et al., 1997; Hess and Carnes, 2004; Shittu, 2006, 2010; Shittu et al, 2007a) and that any disruption of these receptors due to structural abnormalities in the efferent ductules or epididymis will lead to impairments of male fertility in mice (Hess, 1997; Hess & Carnes, 2004; Shittu, 2006, 2010).

Previous studies have also shown that the sectional tissues of studied organs are usually subjected to compression effect by 83% of their original dimension during their tissues processing stages, thereby necessitating the need of a correction factor. However, volume densities are not affected by this compression (Mouton, 2002; Shittu, 2006, 2010; Shittu et al, 2008b; Shittu et al, 2009) and hence, appeared to be more suitable for the present study.

In addition, the respective differential epididymal weights changes observed in the present study as seen in table 6 were well correlated with the tubular profiles of the epididymis especially, the tubular diameter for each group of animals as reflected in table 5, which further goes to support the above stated findings.

Moreover, the efferent ductile or epididymis is also rich in androgen receptors, the site of action for the testosterone (TT) and dihydrotestosterone (DHT) (Deslypere et al., 1992; Grino et al., 1990; Oliveira et al., 2003; Van-dekerckhove et al., 2000).

Contrary to speculation that phytoestrogen can disrupt and cause deleterious effects on the male reproductive developmental organs (Degen and Metzler, 1987). Our previous studies on *sesamum radiatum* leaves phytoestrogens have shown that sesame can indeed stimulate and enhance the release of quality matured spermatozoa from the testis into the epididymes as observed in the photomicrographs with evidence of matured spermatozoa fullness in the dilated epididymal lumens compared to the control group as shown in figure 1-3 (Shittu, 2006; 2010; Shittu et al, 2007a; Shittu et al, 2008a, Shittu et al, 2009) and this was also corroborated with significant ($P < 0.05$) higher epididymal tubular diameters observed in the sesame- treated groups compared to the control group as reflected in table 5b..

Moreover, various other reports are found confirming the epididymal presence of aromatase in human efferent ductules and proximal epididymis (Carpino et al., 2004) and cultured rat cells, (Wiszniewska, 2002). Thus, sesame phytoestrogenic lignans tend to promote aromatization of testosterone to estradiol, such that, the low dose sesame will make available less endogenous estradiol and compete less, although there is synergism at this level between the testosterone and estradiol to favour spermatogenesis. However, the high dose, which will cause more estradiol production and compete more with dihydrotestosterone for aromatization to occur in its favour (Shittu, 2006, 2010; Shittu et al, 2007a; Shittu et al, 2008a; Shittu et al, 2009).

In addition, from the present study, it is postulated that sesame acts through mechanisms, which are dependent on the estrogens receptors (ER1 and ER2) binding and also cause estradiol (E2)-induced transactivation of the androgen receptor (AR), thereby ultimately influencing the hypothalamic-pituitary-testicular pathway as the case may be as evidenced in this present study and others (Shittu, 2006, 2010; Shittu et al, 2007a; Shittu et al, 2008a; Shittu et al, 2009).

Hence, we also hypothesized that the agonistic action of sesame radiatum leaves extract on the α -estrogens receptors is more pronounced than the β -estrogens receptors and with its rich antioxidative property, all contributed to enhancing spermatogenesis with improve male fertility as evident in present study.

5. Conclusion

The result confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed Sesame leaves extracts against common skin infection and bacterial including yeast that are associated with infertility cases in this part of the world. In addition, Sesame leaves extract consumption enhances the quality of the spermatozoa produced with improvement in the storage capacity of the epididymes for these spermatozoa in a dose related manner.

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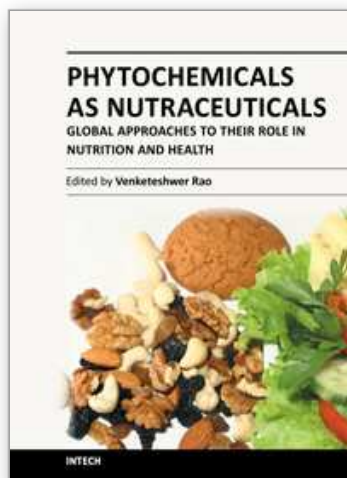
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Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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