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Drug Discovery

Tabletability and sperm boosting properties of *Allium sativum* (garlic), *Tetracarpidium conophorum* (walnut) and *Cyperus esculentus* (tiger nut) powders

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ABSTRACT

Decoctions made with garlic, walnut and tiger nut powders have been used to treat male infertility. In this study, the powders were characterized, formulated into tablets and assessed for their efficacy in treating male infertility through comparison with tablets made from sperm enhancing BF® powder. Characterization of garlic, tiger nut and walnut powders was done by measuring their density, flow, compressibility, sedimentation, viscosity, porosity, swelling, photomicrography and FTIR values. The powders were granulated and compressed into tablets containing only BF® (BFHTs), garlic (GHTs), tiger nut (THTs), walnut (WHTs) or equal ratios (CHTs) as the active ingredient, respectively and assessed using crushing strength, friability and release properties (in phosphate buffer, 0.1N HCL and distilled water) as criteria. Thirty-six adult male Wistar mice were assigned into groups A to K and dosed for 60 days before sperm cell analysis after euthanasia. Groups A to E received 500mg/kg/day and F to G received 1000mg/kg/day of GHTs, THTs, WHTs, CHTs and BF. Group K received distilled water (0.5mL/kg/day). ANOVA and LSD were used to analyze all the data collected. Tiger nut powder exhibited the highest densification at zero pressure (0.48 ± 0.01). Powder flow properties were poor (>50^0), while the compressibility profiles ranked garlic greater than tiger nut and walnut. Garlic powder exhibited the highest sedimentation (41.67±0.13), viscosity (8.50±0.01cps), porosity (24.54±0.04%) and swelling (2.46±0.01) indices. Powders had irregular shapes with varied functional groups, but with similar peaks between 1243.66-1234.00cm^{-1} due to C-O stretching. Crushing strength ranked GHTs>THTs>CHTs>BFHTs>WHTs. CHTs tablets were the most friable and exhibited the fastest disintegration (19.05±1.03secs) and release (>92.0%/60min) profiles. Sperm motility ranked CHTs (92.5±3.4%)>WHTs=THTs=BF(85.0±0.1%)>GHTs(80.0±0.2%), with group D having the highest normal sperm cells (91.5%). Sperm quantity of the mice were comparable, averaging 400.01±1.06 counts. Superior tablet qualities were demonstrated by garlic powder compared to other powders. Low doses of tablets containing the combined

powders displayed qualities comparable to BF®, which could be utilized for treatment of male infertility.

Key words: Tabletability, Garlic powder, Tiger nut powder, Walnut powder, Sperm motility

1. INTRODUCTION

Infertility has been described as the inability to attain conception after 1 year of constant unprotected sexual intercourse (Zegers-Hochschild et al., 2009) Couples unable to conceive have recognizable reasons in about 85% of cases, while the term *unexplained infertility* arises in the remaining 15% of infertile couples, and typically, the commonest causes of infertility are male factor infertility, tubal disease and ovulatory dysfunction (Carson & Kallen, 2015). Agarwal et al. (2015) documented that Southeast Asia and Sub-Saharan Africa comprise the two major areas where infertility is most prominent (Mascarenhas et al.,2012). As of 2010, there were 48.5 million infertile couples worldwide, out of which 20-30% of the cases were due to male infertility (Mascarenhas et al., 2012). A unique view of male infertility across the globe attributed male factor to be responsible for 20-40% of multiple factors involved in infertility (Lunenfeld & Van-Steirteghem, 2004). Male infertility can be evaluated using a comprehensive medical history, a focused physical examination, and particular testing. Infertility may be managed with empirical or targeted medical therapy, life modification, and surgery that results in a discernible increase in fertility (Eisenberg et al., 2023). Various factors such as smoking, consumption of alcohol (Amor et al, 2022), and other environmental elements have negative impacts on male fertility leading to adversely affecting male reproductive functions (Wdowiak et al., 2024). Inadequate quantities (azoospermia or oligospermia), poor motility, and aberrant structure or morphology are abnormalities in spermatozoa that have been recognized as leading causes of infertility in men globally (Sharma et al., 2013) According to reports, only a small percentage of male factor infertility can be resolved with primary methods of treatment, and secondary measures like artificial insemination, intrauterine insemination, *in-vitro* fertilization and embryo transfer, intra-cytoplasm sperm transfer, and child fostering or adoption are used to resolve male infertility (Okonofua et al., 2022).

Increase in male infertility in Africa, particularly Nigeria, the most populous nation in the continent (Pontianus & Oruonye, 2021), has created an urgent demand for the production of natural healing and affordable herbal formulations that are standardized for treating male infertility (Uadia & Emokpae, 2015). Among the typical reactions to infertility are shock, sadness, despair, anger, frustration, a decline in confidence and self-worth, and a general lack of control. For both partners, the pressure of infertility can lead to emotional and psychological distress, including financial challenges. Despite not being a life-threatening condition, infertility can cause a lot of stress, which may be related to the societal value placed on having children (Simionescu et al., 2021).

The use of natural products as alternative remedies for male infertility treatment has been well reported (Nguyen-Thanh et al., 2024). Garlic (*Allium sativum*, Family: *Amaryllidaceae*) improved testosterone levels in mice, which was linked to the presence of s-allyl cysteine (Rana et al, 2021). African walnut (*Tetracarpidium conophorum*, Family: *Euphorbiacea*) is native to southwest region of Nigeria, has been widely cultivated for its delicacy (Nwaichi et al., 2017), and is also used as a fertility agent in males (Folake & Risikat, 2023), due to the presence of vitamin E and a variety of antioxidants in the seeds (Dada & Aguda, 2015). Milk obtained from tiger nut (*Cyperus esculentus*, Family: *Cyperaceae*) produced a dose-dependent increase in sperm count and motility of animals (Ogbuagu & Airaodion, 2020). Due to the side effects of conventional medicines and the newer methods of conception, such as *in-vitro* fertilization, surrogacy, intrauterine insemination, which are not readily affordable, it is reasonable to develop indigenous herbal formulations that can boost sperm production, increase libido, enhance sperm parameters and ultimately treat male infertility. This research focused on assessing the tabletability of powders derived from garlic, walnut and tiger nut, as well as assessing their effectiveness in treating male infertility based on data obtained from animal models. The tablets were compared with a regulatory approved commercial product indicated for the treatment of male infertility (BF).

2. MATERIALS AND METHODS

2.1. Materials

Freshly harvested cloves of garlic, seeds of African walnut and seeds of tiger nut were purchased from registered farmers in Ibadan, southwestern, Nigeria. The products were authenticated at the University of Ibadan herbarium, Oyo state, Nigeria., with identification number UIH-23554, UIH-23556 and UIH-23552 respectively. The reagents and equipment used were acquired from the laboratories of

the Departments of Pharmaceutics and Industrial Pharmacy, Pharmacognosy and Pharmaceutical Chemistry, University of Ibadan, Nigeria.

2.2. Methods

2.2.1. Preparation of garlic, walnut and tiger nut powders

The African walnut seeds were thoroughly rinsed with distilled water, de-shelled and powdered using the blender with model number (MX-AC 400, Panasonic India) and dried in the oven (Gallenkamp BS oven 250 size 1, Leicestershire, United Kingdom) set at 40±0.5°C for 24 hours. The dried African walnut powder was stored in an airtight container. The garlic cloves were thoroughly rinsed with distilled water, peeled, sliced finely and dried in the oven (40±0.5°C) until the cloves became crispy. The crispy cloves were powdered using the blender and transferred into an airtight container. The tiger nut seeds were immersed in distilled water for a period of 4 hours and subsequently blended. The resulting liquid was decanted, and the slurry was filtered using calico cloth. The slurry was then dried in the oven at 40 ± 0.5°C for a duration of 24 hours, after which the dried powder was transferred into an airtight container. The contents of BF® capsules, a commercially approved product indicated for the treatment of male infertility, were emptied into an airtight container.

2.2.2. Characterization of powders

2.2.2.1. Determination of angle of repose

Approximately 10 grams of each powdered sample were poured through a short stem funnel, which was firmly fitted on a retort stand, into an open-ended glass cylinder at an angle of 90°, with a round cork of radius, r, at its base. The open-ended glass cylinder was removed vertically, allowing the powder to flow out and cascade into a heap. and the height of the resultant cone formed was measured using a pair of dividers and a ruler. The angle of repose (θ) formed between the slant height of the cone and its horizontal base was calculated using the equation

$Tan\ \theta = \frac{h}{r}$ Where: h = height of the powder heap (cone), r = radius of circular base. The determinations were made in quadruplicates (Ayorinde et al., 2013).

2.2.2.2. Particle density determinations

The true densities of the powdered samples were determined using the solvent pycnometer method with xylene as the non-solvent. The weight of the empty pycnometer with a capacity of 50 mL (W) was noted, it was then filled with xylene, and the excess xylene was wiped off. The weight of the pycnometer and xylene (W_1) was then determined and the difference in the weight was calculated as W_2. Precisely 2g of the powder sample was weighed (W_3) and transferred into the pycnometer bottle using a funnel. Any excess xylene was wiped off the pycnometer bottle and the bottle was reweighed (W_4). The particle density was then calculated in triplicate using the following equation:

Particle density (gcm^{-3}) = (W_2 x W_3) ÷ 50(W_3 – W_4 +W_2+W)

2.2.2.3. Bulk and Tapped densities

Precisely 10 g of each powder sample was poured into a 50 mL measuring cylinder and the volume (Vp) occupied by the powder was recorded, subsequently, the cylinder was tapped 100 times taps at consistent intervals, and the new volume (Vpt) was recorded. The bulk and tapped densities were calculated as the ratio of the mass of the powder (Mp) to Vp, and to Vpt respectively. All determinations were performed in triplicate.

2.2.2.4. Determination of Hausner's ratio and Carr's Compressibility index

The values obtained from the bulk and tapped densities were used to calculate the Hausner's ratio (HR) and Carr's compressibility index (CCI) using the formulas below (Adetunji et al., 2015):

$HR = \frac{TD}{BD}$ and $CCI = \frac{TD-BD}{BD}$ X 100

Where BD = Bulk density and TD = Tapped density

2.2.2.5. Determination of Sedimentation Volume and Swelling Capacity

Exactly 5g of powder sample was poured into a 100 mL measuring glass cylinder and the volume occupied by the powder was recorded (V_0). Subsequently, a 5% w/v suspension of the powder in distilled water was prepared and allowed to stand for 24 hours prior to measuring the sedimentation volume (SV) in mL. The swelling capacity (SC) and SV were then computed using the equation below (Okunlola, 2015):

$SV = SC \times V_0$

2.2.2.6. Determination of Powder porosity

The porosity of the powder samples was derived from the equation below (Adeleye et al., 2015)

$E = 1 - \frac{BD}{TD} \times 100$ Where BD = Bulk density and TD = Tapped density

2.2.2.7. Fourier Transform Infrared Spectroscopy (FTIR)

The powder samples were analyzed by FT-IR (FT-IR Spectrum BX II by PerkinElmer, Waltham, MA, USA) in transmission mode at a spectra range of 4000 - 400 cm-1 using 64 scans with resolution of 8 cm-1 (Ayorinde et al., 2013).

2.2.2.8. Determination of Surface Morphology (Photomicrography)

The photomicrograph of each powdered sample was captured using a light microscope installed with a calibrated eye piece. Subsequently, the surface morphology was analyzed using a desktop scanning electron microscope (FEI-XL 30SEM, Phenom World, Netherlands).

Table 1: Formulations (%) used for granulation and tablet compression

Code	BF Powder	Garlic powder	Walnut powder	Tiger nut powder	Corn starch	Lactose Powder	Tracer Dye (0.67%)
BFHTs	50.00	-	-	-	12.50	36.83	Green
THTs	-		-	50.00	12.50	36.83	Blue
WHTs	-	-	50.00	-	12.50	36.83	Red
GHTs	-	50.00	-	-	12.50	36.83	Yellow
CHTs	Equal proportions of THTs, WHTs and GHTs						

BFHTs: Herbal tablets containing granules of BF® as the active component
THTs: Herbal tablets containing tiger nut granules as the active component
WHTs: Herbal tablets containing walnut granules as the active component
GHTs: Herbal tablets containing garlic granules as the active component
CHTs: Herbal tablets containing equal proportions of tiger nut, walnut and garlic granules as the active component.

2.2.3. Preparation of Granules and Tablet Compression

Formulations containing predetermined quantities of garlic powder (or African walnut powder or tiger nut powder) and other excipients as shown in Table 1 were dry mixed for 15 minutes in a planetary mixer (Model A120, Hobart Manufacturing Co., U.K.) and moistened with appropriate amount of paste of the binding agent (corn starch) to produce samples containing different concentrations of the binder. Massing was continued for about 5 minutes, and the wet masses were granulated by passing them through a sieve (1,400 μm). The granules were dried in hot air oven for 16 hours at 40°C. Tracer dyes of various colours were incorporated into the stored granules before compression, and the level of mixing was ascertained using the method suggested by Lamotte (2018). Tracer dyes of various colors were incorporated into the stored granules before compression, and the extent of mixing was determined using the method suggested by Lamotte (2018). The dried granules (containing tracer dyes) were then re-sieved through a sieve (1,000 μm), before they were stored in air-tight containers. The granules were compacted into tablets under a load of 1 metric ton with a Carver hydraulic hand press (Model C, Carver Inc., Menomonee falls, Winconsin, U.S.A) that has a 10.5mm flat faced punch and die set, which has been lubricated with a 1% dispersion of magnesium stearate in acetone before compaction. All the tablets were stored over silica gel

for a duration of 24 hours for elastic recovery to occur and subsequently, avoid false low-yield pressure before the tablets were evaluated (Adetunji et al., 2006).

2.2.4. Evaluation of Tablets

2.2.4.1. Determination of Crushing Strength and Friability

The crushing strength of the tablets was determined in triplicate at 27±2 ^{0}C by means of a tablet hardness tester (DBK Instruments 400-060 model EH 01, Mumbai, India). The results were valid only if the tablets fractured diametrically into equal halves. A friability tester (Shivani Scientific, India) was utilized to conduct the friability test. The equipment was loaded with ten (10) previously weighed tablets (W_1). These tablets underwent rolling and repeated shocks as they fell in each rotation (Adetunji et al., 2015). The tablets were reweighed after 4 minutes of treatment, or 100 rotations, (W_2) and the percentage friability (F) was determined using the formula:

$$\text{Friability } (\%) = \frac{(W1 - W2)}{W1} \; x \; 100$$

2.2.4.2. Disintegration test

The disintegration test was carried out using a Veego tablet disintegration apparatus set at 37.0±0.5 ˚C. The time it took each tablet to completely pass through the basket was recorded. The determinations were carried out in triplicate (Adetunji et al., 2015)

2.2.4.3. Dissolution test

The dissolution test on the tablets was conducted using the USPXX III basket method (DBK Dissolution rate test apparatus Model 001, Mumbai, India). The device was set to rotate at 100 rpm at a temperature of 37.0±0.5 ˚C and contained 900 ml of 0.1N hydrochloric acid using the set to rotate at 100 rpm. Samples (5 mL) were withdrawn at designated intervals and replaced with equal amounts of fresh dissolution medium. The absorbance values of the samples were recorded using a UV/visible spectrophotometer (Spectrum Lab 752s, Shanghai, China) and the total concentration of the active component of each tablet was calculated (Okunlola, 2015).

2.2.5. Experimental Animals

A total of 33 adult male Wistar mice, each weighing between 19 g and 23 g were randomly divided into 10 groups (A,B,C to J) that received doses and a control group (K). The animals were acclimatized for 14 days in standard plastic cages located in a well-ventilated room before dosing commenced. Each animal was provided with an intensive nutritional diet programme containing unlimited access to mice grower pellets and distilled water throughout the experiment.

2.2.6. Experimental Design

The tablets were crushed and dissolved in distilled water at a concentration of 12.5%w/v of the active component. Groups A, B, C, D and E received 500mg/kg/day of GHTs, WHTs, THTs, CHTs and BFHTs respectively, while Groups F,G,H,I and J received 1,000mg/kg/day of GHTs, WHTs, THTs, CHTs and BFHTs respectively. Group K animals received 0.5 mL of distilled water. The animals were orally dosed for 46 days and sacrificed on day 61 of the experiment.

2.2.7. Body weight measurement and animal sacrifice

The body weight of the animals in each group were documented every three days. The Wistar mice were anesthetized with 80mg/kg ketamine and 5mg/kg xylazine, before euthanasia by cervical dislocation, which was done 24 hours after the last dose was administered., after they. The testicles were surgically removed, and semen samples were taken from the cauda epididymis (Oyeyemi, 2006).

2.2.8. Tests on Sperm Cells

The extracted sperm cells were tested for morphology, number, motility and viability.

Morphology: The morphology of the sperm cells was determined using the method of Oyeyemi and Ubiogoro, 2005). Exactly 5µL of the epididymal fluid was carefully collected, placed on a glass slide and viewed under the microscope (x 400 magnification) before area

filed scanning of the microscopic field was done. The morphology was classified using the parameters: head without tail, curved mid-piece, abaxial mid-piece, simple bent tail, coiled tail, looped tail, rudimentary tail, coiled around head, tail without head,

Number: To determine the number, the sperm cell solution was passed through an 80µm stainless mesh filter prior to measuring the number of epididymal sperm with the upgraded Neubauer cytometer. The resuls obtained and the values were reported as millions/mL of suspension.

Sperm Motility: The percentage sperm motility was assessed by placing 5µL of the sperm cell solution on a microscope slide, after which the quantity of the increasingly mobile sperm cells was then counted and divided by the total amount of spermatozoa.

Sperm Viability: The Eosin-Nigrosin one-step staining method was utilized in evaluating the sperm viability. Five air-dried smears of each sample were made on glass slides, after which a portion of the sperm solution was combined with an equal volume of Eosin-Nigrosin stain. The slides were examined within fifteen minutes to ascertain their percentage viability. Dead sperm cells absorbed the dye and appeared pink, whereas normal healthy sperm cells rejected it and appeared whitish. (Chukwu et al., 2022).

3. RESULTS

Angle of Repose, Density Measurements, Hauner's ratio and Carr's Compressibility Index

The results obtained for the measurement of the angle of repose and densities (Particle density, bulk and tapped density) for the powders are summarized in Table 2. The highest densification at zero pressure (0.48±0.01) was demonstrated by Tiger nut powder. Among the three powders under investigation, the compressibility profiles were ranked as follows garlic > tiger nut > walnut.

Table 2: Table showing angle of repose, density measurements, Hauner's ratio and Carr's compressibility index for the powdered samples of BF®, Tiger nut, Walnut and Garlic (n=3±SD)

Powder Samples	Angle of Repose (^{0})	Particle Density (gcm^{-3})	Bulk Density (gcm^{-3})	Tapped Density (gcm^{-3})	Hausner's ratio	Carr's Compressibility Index (%)
BF®	50.09±0.13	2.09±1.03	0.70±0.02	0.81±0.02	1.16±0.02	15.71±0.02
Tiger nut	54.72±0.17	1.52±0.13	0.48±0.01	0.59±0.13	1.23±0.07	22.92±0.02
Walnut	50.64±0.02	1.12±0.01	0.38±0.03	0.46±0.04	1.21±0.04	21.05±0.14
Garlic	52.95±0.07	1.53±1.02	0.26±0.13	0.35±0.01	1.35±0.07	34.62±0.24

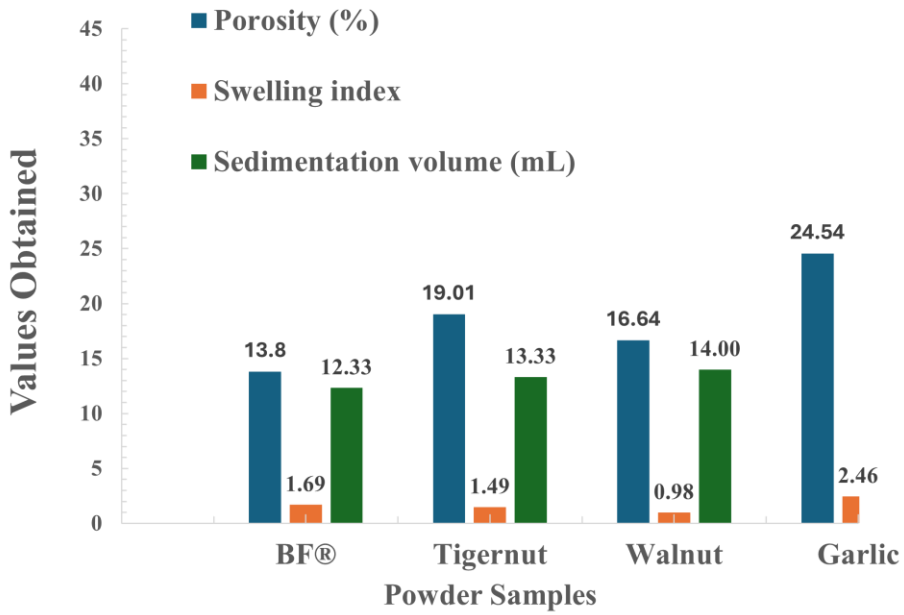
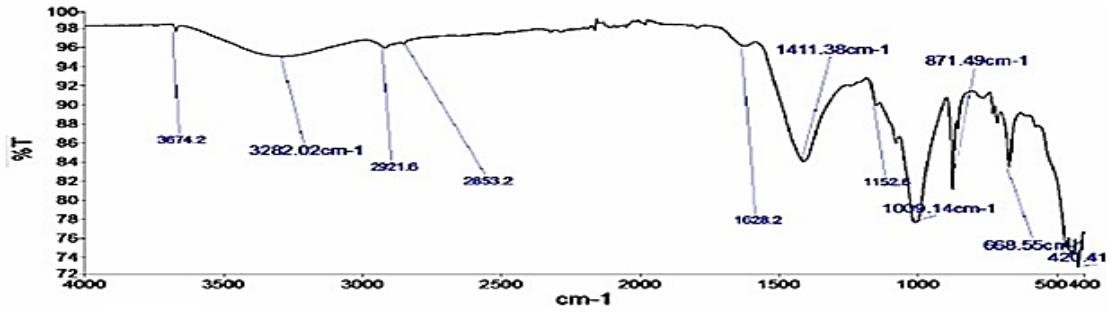
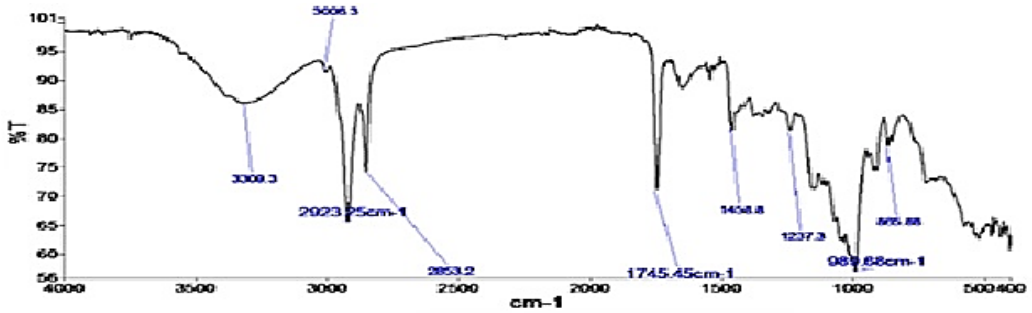


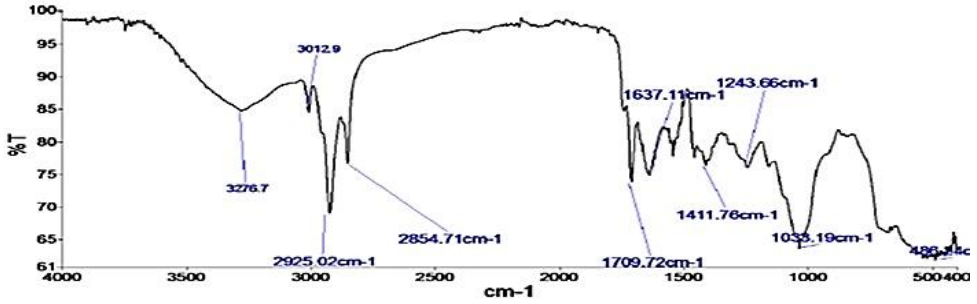
Figure 1: Values (n=3±SD) obtained for the Sedimentation Volume (mL), Swelling Index and Porosity (%)



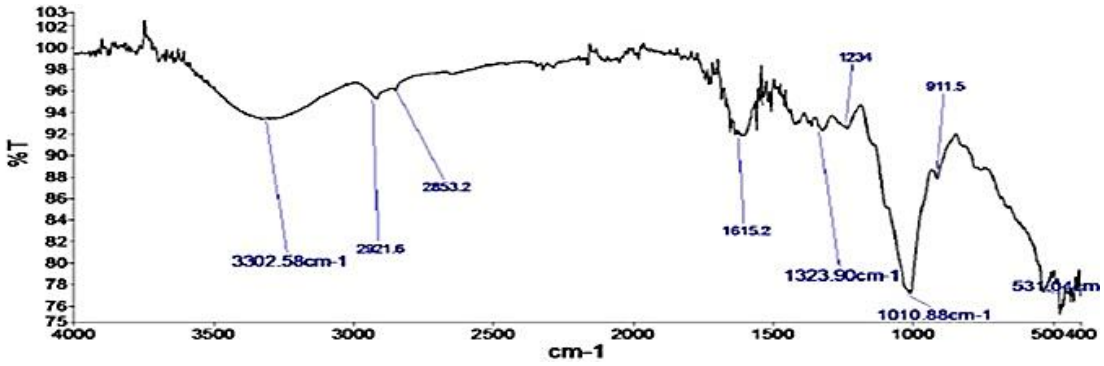
2(a) FTIR Plot for BF® Powder



2(b) FTIR Plot for Tiger nut Powder



2(c) FTIR Plot for Walnut Powder



2(d) FTIR Plot for Garlic Powder

Figure 2: FTIR Plots for powder samples

Sedimentation Volume (mL), Swelling Index, Porosity (%) and Viscosity (cps)

The sedimentation volume (mL), swelling index and porosity (%) of the powder samples are presented in Figure 1, while the viscosity values from three determinations are summarized in Table 3. Amongst the samples, garlic powder exhibited the highest sedimentation (41.67±0.13), viscosity at 100 cps (8.50±0.01cps), porosity (24.54±0.04%) and swelling (2.46±0.01) indices.

Table 3: Values obtained for the viscosity (cps) at 37.0± 0.020C

Powder samples	Viscosity (cps) at 20 rpm	Viscosity (cps) at 50 rpm	Viscosity (cps) At 100rpm
BF®	undeterminable	3.00±0.00	7.00±0.01
Tiger nut	undeterminable	3.00±0.00	7.50±0.11
Walnut	2.50±0.03	3.00±0.00	7.00±0.14
Garlic	2.50±0.01	5.00±0.02	8.50±0,01

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 2 shows the FTIR plots for the powder samples. Tiger nut and walnut powders exhibited plots that are similar at the fingerprint regions, while all the three powders under investigation showed different functional groups, but similar peaks between 1243.66 and 1234.00 cm^{-1}.

Photomicrograph

The surface morphology of the powder samples examined at a magnification of 400 is shown in Figure 3. The powders exhibit irregularly shapes; however, the average surface sizes were determined to be BF® powder (61.25µm), tiger nut powder (550µm), walnut powder (1,150µm) and garlic powder (550µm).

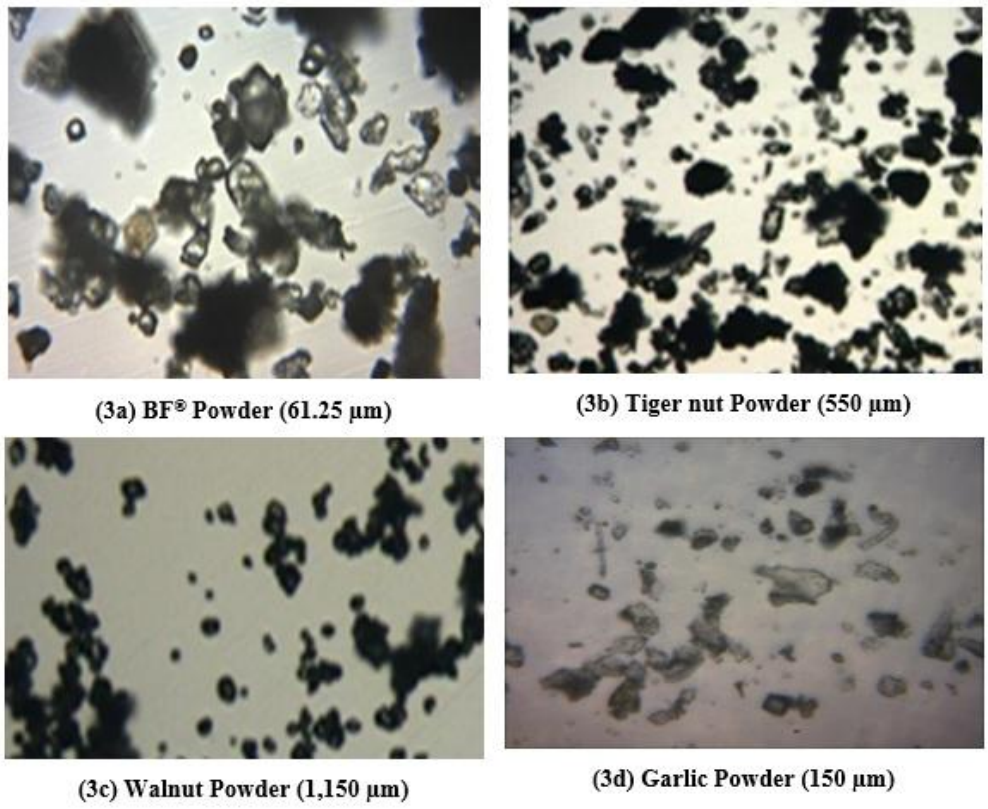


Figure 3: Photomicrograph of powders showing the surface morphology and calculated average diameter (µm) (X 400 magnification)

Table 4: Crushing strength, friability and crushing strength-friability ratio (CSFR) for tablets

Code	Crushing strength (N)	Friability (%)	CSFR	Disintegration Time (secs)
BFHTs	8.42±0.12	1.02±0.11	8.25	22.19±0.18
THTs	16.71±0.04	0.81±0.01	20.63	50.58±0.27
WHTs	8.03±0.17	0.59±0.03	13.61	25.49±0.04
GHTs	41.67±0.04	0.52±0.01	80.13	23.03±0.13
CHTs	9.63±0.16	3.75±0.03	2.57	19.05±1.03

Crushing Strength, Friability, Disintegration Time and Dissolution of Herbal Tablets

The crushing strength, friability, crushing strength-friability ratio and disintegration time for the herbal tablets are summarized in Table 4, while their dissolution plots in different media are illustrated in Figures 4-6. Crushing strength values were ranked as GHTs >THTs > CHTs > BFHTs > WHTs. Tablets containing the combined powders (CHTs) were the most friable and demonstrated the fastest disintegration (30.05±0.16 secs) and release profiles (>92.0% in 60 min).

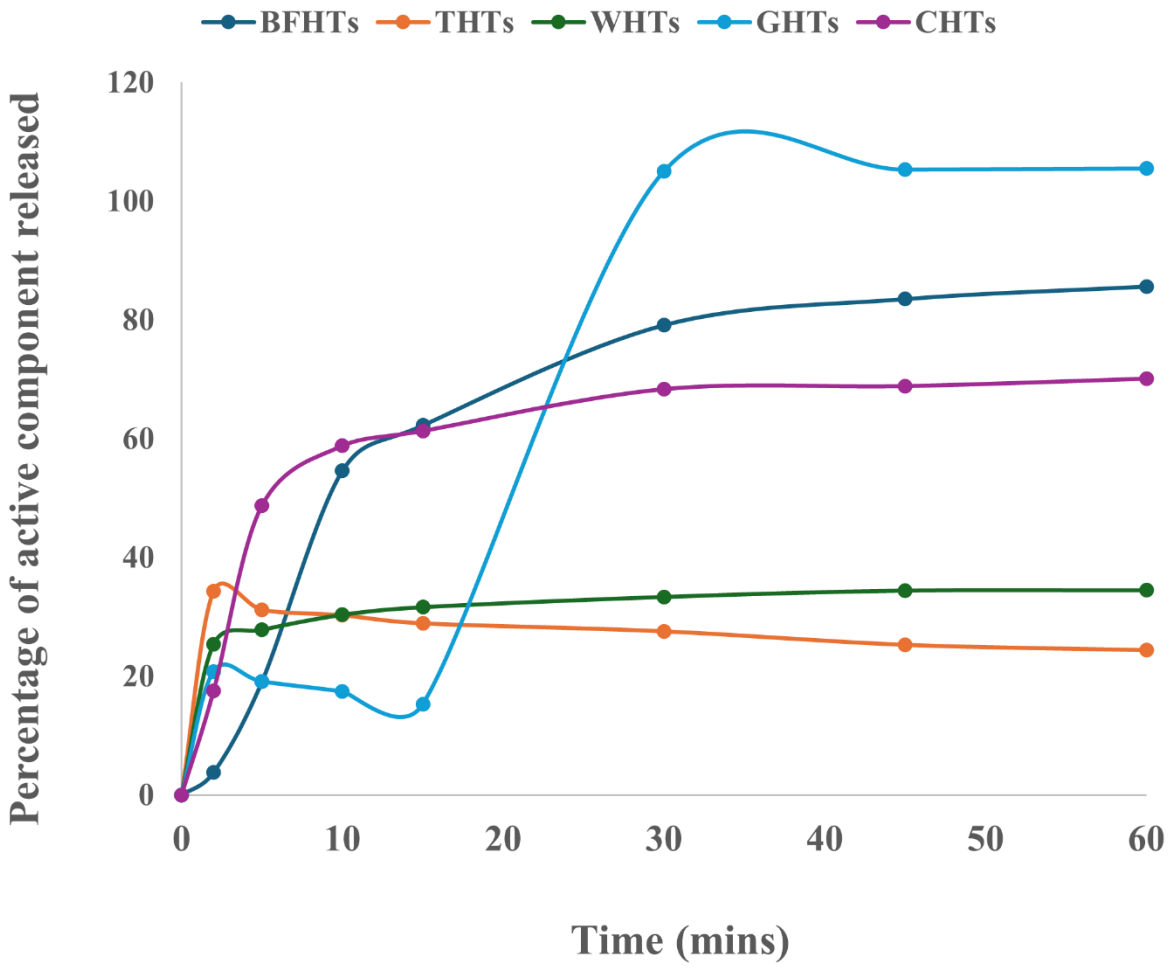


Figure 4: Dissolution profile of herbal tablets in distilled water (37±0.5 ^{0}C)

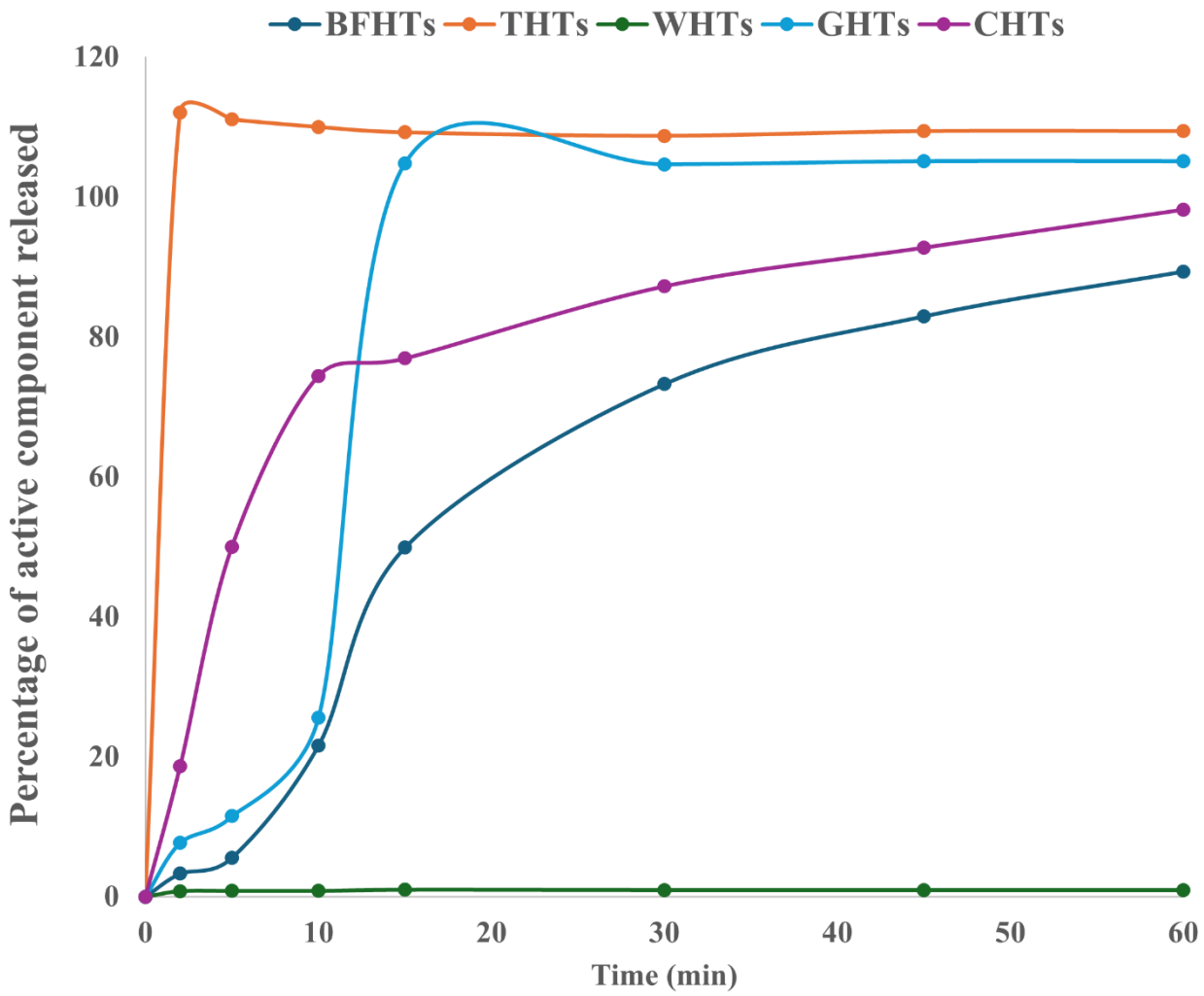


Figure 5: Dissolution profile of herbal tablets in phosphate buffer (37±0.5 ^{0}C)

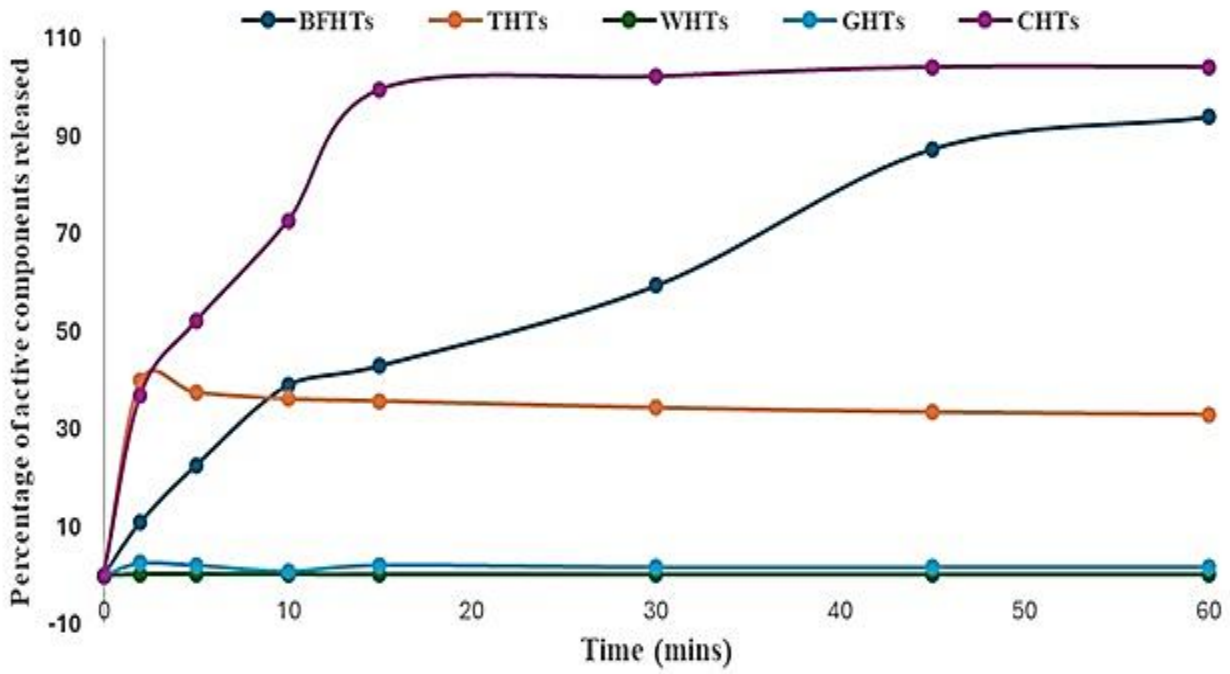


Figure 6: Dissolution profile of herbal tablets in 0.1N HCL (37±0.5 ^{0}C)

Morphology, Number, Motility and Viability of Sperm Cells

The morphology of the sperm cells for the respective groups is illustrated in Table 5, whereas the normality, motility and viability of the sperm cells are shown in Figure 7.

Table 5: Morphology of the sperm cells for the groups

Group	HWT	CMP	AMP	SBT	CT	LT	RT	CAH	TWH
A	9	10	0	11	3	9	0	3	10
B	10	10	0	10	3	6	0	1	7
C	9	8	0	9	7	8	0	4	7
D	7	6	0	7	5	3	0	1	5
E	11	11	0	11	10	10	0	4	9
F	11	8	0	9	4	10	0	4	11
G	10	9	0	10	5	7	0	2	8
H	10	10	0	11	9	8	0	4	8
I	9	9	0	8	7	4	0	1	5
J	12	12	0	9	9	11	0	4	11
K	6	5	0	6	2	2	0	2	7

HWT= Head without tail, CMP = Curved mid-piece, AMP = Abaxial mid-piece, SBT = Simple bent tail, CT = Coiled tail, LT= Looped tail, RT = Rudimentary tail, CAH = Coiled around head, TWH = Tail without head

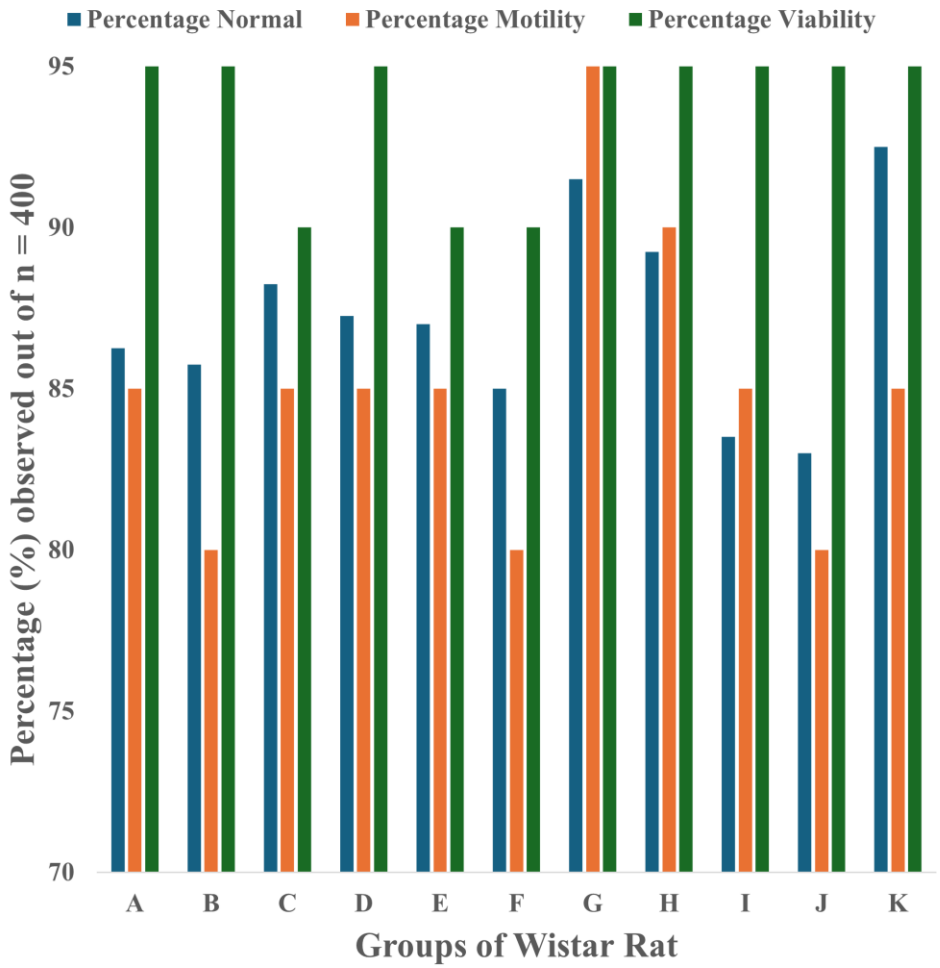


Figure 7: Plot showing the percentage of sperm cells that are normal, motile and viable out of the total number of 400 sperm cells

4. DISCUSSION

Powders of garlic, walnut and tiger nut have been utilized as decoctions to treat male infertility due to their easy accessibility, cost effectiveness and biodegradability. Efforts to improve the presentation of the powders led to their characterization, compression into tablets and assessment of their efficacy in addressing male infertility. The compressed tablets were then evaluated and compared with a commercial product indicated for the treatment of male infertility (BF®).

Particle density quantifies the compaction properties of a powder, which is essential for processing parameters including compaction, tooling design, and press motion requirements (Apeji et al., 2013). Powders tend to segregate due to factors such as differences in particle size, density shape and durability. Based on their particle densities, the powders were ranked as follows: tiger nut > garlic > walnut>BF®, therefore indicating that BF® and walnut powders will require high compression pressure during the process of tableting. Bulk density quantifies the densification of powders at zero pressure, whereas tapped density is a measure of a powder's packing characteristics, which can affect the storage, compaction and flow of the powder (Santomaso et al., 2003). Tiger nut powder had the highest densification at zero pressure (0.48±0.01), implying that it demonstrated the highest possibility of clumping up or segregation on storage. This prediction is also confirmed in the result of the angle of repose, where tiger nut powder had the highest value ($54.72±0.17^0$) and is the most resistant to flow.

A direct relationship exists between the cohesive nature of a powdered material and angle of repose (Uzondu et al., 2014). Poor flowability indicates that the powders possess strong interparticulate attractions which can lead to their consolidation and subsequently

obstruct the orifice of the hopper during the formation of tablets (Rahim et al., 2014). Apeji et al (2013) classified angle of repose of 30° and below as indicative of free-flowing powder, while angles between 30°- 40° are considered passable, and angles exceeding 40° are considered as highly resistant to flow. All the powders demonstrated high resistance to flow with angle of repose greater than 50°. Similarly, Carr's compressibility index (CCI) assesses the compressibility of powders, providing insights into the flow and inherent behavior of powders throughout the tabletting process (Freeman & Price, 2009). Low CCI values give good flowability, but poor compressibility. Riley & Adebayo (2010) classified CCI values of 5-10, 12-16, 18-21, 23-35 as excellent, good, fair and poor flow respectively. The degree of densification that may occur due to dynamic motion when powders are introduced from the hopper during the tabletting process can be estimated using Hausner's ratio (HR) (Adetunji et al., 2015). A low HR value affirms that die filling will be significantly favoured during the process of tabletting. Among all the powders, walnut powder demonstrated the lowest values of CCI (21.05±0.14 %) and HR (1.21±0.04), indicating the tendency of good die filling processes.

The sedimentation rate of a powder is the speed at which the powder settles or is removed from a medium, such as water, air or fluids. Garlic powder exhibited the highest sedimentation value (41.67mL) out of all the powders, indicating that it has the highest tendency to settle quickly in water. The swelling index measures the amount of water that can be absorbed by a powder and can be used to predict the release property of a material during the process of dissolution. Consequently, a low swelling index indicates that the material will likely hinder drug release (Chen et al., 2015). Garlic and walnut powders showed the highest (2.46±0.06) and lowest (0.98±0.11) swelling indices, respectively.

Porosity is a key attribute of powders that can be used to predict the behaviour of tablet formulations during disintegration and dissolution tests (Odeniyi et al., 2011). A powder characterized by high porosity will enhance solvent penetration and subsequent liberation of the active components during the tableting process. The extent of volume reduction observed when pressure is exerted on a powder bed is also a way of measuring compressibility, which is interrelated to porosity. Garlic powder, with a value of 24.54±0.01, had the highest porosity value.

The resistance of a powder to gradual deformation because of shear or tensile stress is an indicator of its viscosity (Olayemi et al, 2021). The viscosities of the powders were measured at three shearing rates (20, 50 and 100 rpm). At the three shearing rates used, garlic powder demonstrated the highest value, which increased gradually to 8.50±0.01cps at the shear rate of 100 rpm. This suggests that garlic powder could serve as a good material in formulations where reduction in viscosity is desired.

The FTIR plots exhibited various differences due to the distinct functional groups in the powder materials, However, N-H stretching (amine salt) and O-H stretching (carboxylic acid) were observed in all the powders, along with similar peaks between 1243.66-1234.00 cm^{-1} due to C-O stretching.

The photomicrographs of the powder samples indicate irregular shaped particles with no specific conformity. Particles that are spherically shaped have demonstrated good flowability, and therefore the goal of ensuring good compressibility during the tableting process is to incorporate spherically shaped particles. (Odeniyi et al., 2011). This indicates why the powders were granulated before compression into tablets.

The majority of herbal formulations that have received regulatory approval for the treatment of male infertility are offered in capsule form. Nevertheless, tablets continue to be the most commonly produced solid dosage form, primarily due to their ease of formulation and widespread acceptance (Adetunji et al., 2006) Tiger nut, walnut and garlic powders were granulated along with the other excipients and compressed into tablets according to the formula in Table 1.

The mechanical properties of the tablets were evaluated by crushing strength and Friability tests that assessed the mechanical strength and measure of tablet weakness respectively. Crushing strength ranked the tablets GHTs >THTs > CHTs >> BFHTs > WHTs, with CHTs observed as the most friable of all the tablets (Table 4). Another parameter for measuring tablet strength is the crushing strength-friability ratio (CSFR). Generally, tablet strength has a direct relationship with the CSFR value. The tablet containing garlic as the active component had the highest CSFR and therefore, has the highest mechanical strength, which is also supported from the ranking of the crushing strength value as it required the highest load (41.67±0.04 N) to cause it to fracture.

The release properties of the tablets were assessed using the disintegration and release properties (assessed in distilled water, phosphate buffer and 0.1N HCL as dissolution media). All the tablets met the British Pharmacopoeia specifications for disintegration of uncoated tablets within 15 minutes. The friability test result is also in concert with the disintegration test results as all the tablets (except CHTs) were within the recommended friability value of 1% or less. The results of the dissolution tests in different media did not follow any specific pattern as the tablets exhibited different release patterns in the different media. The media were carefully selected to

represent acidic (0.1N HCL), basic (phosphate buffer) and neutral (distilled water) conditions, which can aid in advancement of formulation development, particularly when targeted delivery formulations are involved. In distilled water, only GHTs and BFTHs released 50% of the active components within 10 minutes and were the sole formulations to exceed 80% release of the active component, with GHTs achieving a complete 100% release within 30 minutes. In the acidic medium, CHTs demonstrated the most favourable release profiles, releasing more than 50% and 80% of the active component within 5 and 30 minutes respectively, closely trailed by BFHTs (after 30 and 45 minutes respectively). GHTs that showed excellent release profiles in distilled water performed poorly in 0.1N HCL. For the phosphate buffer, there was an outburst of active component release from THTs (over 100% released within 2 minutes), which might be a potential consideration for emergency situations. However, GHTs showed promising results in phosphate buffer, closely followed by CHTs and BFTHs. Among all the herbal tablets under investigation, only CHTs exhibited reliable release profiles in all the media used for dissolution.

For adequate fertilization to occur, sperm cells must have well-formed head (for fusing with the egg) and tail (for adequate motion to swim up the fallopian tube and reach the egg). A tightly packed helical arrangement of mitochondria within the mid piece of the sperm delivers energy necessary for the propulsion of the cell; the quantity of the mitochondria influences the frequency of flagella movements. Consequently, the outcome of sperm competition may be affected by the size of the sperm midpiece. Comparative research among vertebrates has in fact demonstrated a favorable correlation between sperm size and swimming speed. (Firman et al. 2010). The percentage of mobile sperm is a major factor in fertility rates and embryologists use sperm motility to assess male fertility, select sperm for optimal use, and maximize the success of assisted reproductive technologies (Goodson et al. 2011). Among the groups that were dosed, group G that were administered with 1000mg/kg/day of walnut had the highest percentage (92%) of normal sperm cells, highest motility (95%) and highest viability (95%) while group J (fed with1000mg/kg/day of BF®) had the lowest percentage of normal sperm cells (83%) and motility (80%). The different morphology of the sperm cells has summarized in Table 5; however, it is worth mentioning that none of the sperm cells exhibited abaxial midpiece or rudimentary tail.

5. CONCLUSION

The tabletability of powders derived from garlic, walnut and tiger nut have been confirmed, however, garlic powder demonstrated superior tabletability profiles than others. The effectiveness of the powders in treating male infertility has also been demonstrated, however, it is crucial to understand the site of action where tablets containing the individual powders will best act. At lower doses, tablets containing the combined powders demonstrated potential pharmaceutical properties similar to BF®, which could be utilized in the treatment of male infertility.

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Authors Contribution

This work was carried out in collaboration between all authors. Author Adetunji OA and Ashiru A designed the study, author Ashiru A carried out statistical analysis and conducted the actual laboratory work. Authors Adetunji OA and Ashiru A wrote the manuscript and managed the literature searches, author Itiola OA played supervisory role. All authors read and approved the final manuscript.

Ethical Approval

The Animal ethical guidelines are followed in the study for observation, identification & experimentation. Ethical approval for this study was obtained from the Animal Care and Use Research Ethics Committee, University of Ibadan, Ibadan, Nigeria, and was assigned the approval number: NHREC/UIACUREC/05/12/2022A. Also, the ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation.

Informed Consent

Not applicable

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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