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# Membrane stabilization and andro-spermatogenic potential of standardized fraction of *Cyperus esculentus* L.

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This study evaluated the membrane stabilising and aphrodisiac potentials of *Cyperus esculentus* L. in male rats. The membrane stabilization effect of the fraction was demonstrated against rat erythrocytes. For the aphrodisiac study, rats were randomized into five groups with animals in group 1 given sterile placebo and served as control. While rats in group 2 received 7.14 mg/kg of Powmax M, animals in groups 3, 4 and 5 were given 15, 30 and 50 mg/kg of *Cyperus esculentus*, respectively. Administrations were done once daily for 4 weeks via oral intubation and their testicular parameters were thereafter determined. Significant improvements were observed on the testes-body weight ratio, quality and viability of sperm cells as well as testicular concentrations of proteins, cholesterol, glycogen, testosterone, follicle stimulating hormone and luteinizing hormone following treatment with the fraction. The fraction also had respective membrane stabilization capabilities of 89.25 and 85.71 % on rat erythrocytes against hypotonic solution and heat-induced hemolysis. The data presented in this study have justified *Cyperus esculentus* as a rich source that could be potentially useful for the development of aphrodisiac drugs and also lent credence to its significance in the traditional system of medicine.

**Keywords:** Androgenesis, Erectile dysfunction, Penile erection, Spermatogenesis, Tiger nut, *Cyperus esculentus* L.

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Erectile dysfunction (ED) is defined as a man's recurrent inability to attain and/or maintain penile erection sufficient for satisfactory sexual activity[1]. Globally, studies show a high prevalence and incidence of ED correlating with general dissatisfaction, age and other sexual dysfunctions[2,3]. Orthodox management therapies for ED have been embraced over the years[4]. While their effectiveness is undoubtable, affordability, sensitivity, social stigma (attached to it in the African context) and inherent side effects have undermined their applications[5]. Consequently, medicinal plants through the traditional knowledge are being used globally as alternative therapy[6]. These botanicals are readily available, more affordable, easily accessible and often with minimal side effects. It is, therefore, not surprising that, resort to the traditional knowledge has continued to be a popular choice for men and women seeking to improve their sexual life[7,8].

In Nigeria, *Cyperus esculentus*, a nut/tuber belonging to Cyperaceae family and popularly called tiger nut (tiger grass), is one of several plants commonly used ethnomedicinally in the management of ED[9]. Preliminary phytochemical analysis of its aqueous extract revealed the presence of alkaloids, glycosides, resins, tannins, sterols, and saponins[10]. p-cymol, vincristine, vinblastine, capscicine, thymol, digoxigenin, and digitoxigenin are the major adaptogenic constituents of GCMS analysis of its aqueous extract[11]. Oladele & Aina[12] have also reported *Cyperus esculentus* to be an excellent source of iron, calcium, sucrose, protein and fat. Its pharmacological significance as anti-sickling, anti-leukemic, anti-flatulence, heamatinic, diuretic, laxative, hypolipidemic and anti-diabetic agent have been documented[13,14]. *Cyperus esculentus* can be eaten raw, roasted, dried, baked or made into a refreshing beverage called '*kunnu*' in *Hausa* language and normally served to the newly wedded couples in the Northern part of Nigeria[12]. Though, studies have

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implicated *Cyperus esculentus* to enhance sexual performance[15-17], evidence of its acclaimed ability to arouse sexual desire is just receiving scientific attention. While Allouh *et al.*[18] have previously reported the influence of its powdered formulation on the copulatory behaviour of male rats and attributed the observed effects to its phytochemical constituents, the findings of Ekaluo *et al.*[19] and our earlier report[11] have also associated improved sexual invigoration in experimental male animals to its crude extract. Besides being crude extracts, there is dearth of comprehensive information on the andro-spermato–genetic potential of standardized fraction of *Cyperus esculentus.* Thus, this study was conceptualized with a view to providing substantiated biochemical information on its overall aphrodisiac activity. The potency of *Cyperus esculentus* against hypotonic solution/ heat-induced hemolysis was also investigated.

## Methodology

### Chemicals and reagents

Dimethyl sulfoxide (DMSO) was procured from Sigma-Aldrich Co. (St. Louis, USA) while Powmax M was a product of Beijing Kowloon Pharmaceuticals Co., Limited, Beijing, China. The experimental kits for protein, cholesterol and glycogen were products of Randox Laboratories Limited (Co Antrim, United Kingdom) while those of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were obtained from Monobind Inc. (California, USA). The water used was glass-distilled and all other chemicals and reagents were of analytical grade.

### Plant collection, authentication and processing

Fresh nuts of *Cyperus esculentus* were procured from Emir's market (Oja-Oba), Ilorin, Kwara State, Nigeria and were authenticated at the department of Botany, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (no. UIH/14/21781) was deposited. The nuts were washed and oven dried at 37 °C for 48 h and thereafter pulverized into smooth powder. The powdered sample (2 kg) was extracted with 70 % methanol (10 L) with regular agitation for 24 h. The solution obtained was filtered (Whatman no. 1 filter paper) and the resulting filtrate concentrated to a yield of 652 g crude extract. Part of the crude extract (500 g) was suspended in distilled water (0.7 L) and subsequently partitioned in succession with n-hexane, dichloromethane, ethyl acetate and n-butanol. This yielded 15 g, 22 g, 30 g and 43 g of the respective fractions and about 10 µL of each (1 mg/mL) was spotted on silica gel TLC plate. The resulting chromatograms were thereafter developed in dichloromethane/methanol (8.5:1.5 v/v) system and sprayed with 0.2 % 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol for detection of antioxidant metabolites. From the chromatograms, the ethyl acetate fraction of *Cyperus esculentus* (CEEAF) had the highest number of antioxidant spots and was selected for subsequent bioassays.

### Experimental animals

This study was carried out following approval from the Ethical Committee of Kwara State University, Malete, Nigeria. *Rattus norvegicus* (n = 50 males) with average weight 235.00 ± 9.50 g (18 weeks old) were collected from the experimental animal facility of University of the Free State, and were housed in clean metabolic cages placed in a well-ventilated house with standard condition. They were acclimatized to the animal house condition for 10 days during which they had *ad libitum* access to pelleted rat chow (Pioneer Food (Pty) Ltd, Huguenot, South Africa) and water.

### Experimental protocol

The male rats were randomized into five groups of 10 animals each. Animals in group 1 were given sterile placebo (DMSO) and served as control. While rats in group 2 were treated with 7.14 mg/kg body weight (b.w.) of Powmax M [a reference male sexual stimulant and energy boosting polyherbal drug composed of *Panax ginseng*, *Camelia sinensis*, *Cnidium monnier*, *Epimedium brevicornum*, *Songaria cynomorium*, *Gingko biloba*, *Dahurian angelica*, *Salvia miltiorrhiza* root, L-arginine hydrochloride and Gamma aminobutyric acid as active constituents] [20,21], animals in groups 3, 4 and 5 were administered with CEEAF (in DMSO) at doses of 15, 30 and 50 mg/kg b.w., respectively. All administrations (1 mL) were done once daily for 4 weeks via oral intubation.

### Sperm motility, viability and counts

Twelve hours after the last treatment, the rats were humanely sacrificed under halothane anaesthetization and the method of Salman & Ayoade[22] was adopted in this assessment. Briefly, the caudal epididymis of the rats was dissected. An incision (about 1 mm) was made and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. By computing the motile spermatozoa per unit area, the epididymal

sperm motility was thereafter assessed. The sperm viability was also determined using Eosin/Nigrosin stain. For the sperm count, the epididymis was homogenized in normal saline (5 mL) prior to counting in the counting chamber of haemocytometer.

**Testicular homogenate preparation**

Following anaesthetization, the testes were also immediately but diligently excised from the rats, cleaned and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were centrifuged at 10000 g for 10 min at 4 °C to obtain post-mitochondrial fractions and the resulting supernatant was stored at -20 °C to ensure maximum liberation of the testicular fractions.

**Determination of biochemical parameters**

The testicular cholesterol concentration was determined in accordance with the manufacturer's specification in the assay kit. Standard methods were used in the estimation of the testicular proteins and glycogen[23,24]. The levels of testosterone, FSH and LH in the testicular homogenate were determined by immunoenzymometric assay as detailed in the manufacturer's guide.

**Membrane stabilizing activity**

**Hypotonic solution and heat-induced hemolysis**

The methods of Sabiu & Ashafa[25] were adopted in these experiments and the respective degree of inhibition against hypotonic and heat-induced lysis or membrane stabilization was calculated using the expression:

$$\% \text{ inhibition of hemolysis} = 100 \text{ X } (A_c - A_s / A_c)$$

where; $A_c$ = Absorbance of control (hypotonic-buffered saline solution alone) and, $A_s$ = Absorbance of test sample in hypotonic solution, and

$$\% \text{ inhibition of hemolysis} = 100 \times (A_H - A_U / A_c - A_U)$$

where; $A_H$ = Absorbance of test sample heated, $A_U$ = Absorbance of test sample unheated and,

$A_c$ = Absorbance of control sample heated.

**Quantitative phytochemical constituents' determination**

Phytochemicals analyses for the detection and quantification of bioactive principles in the ethyl acetate fraction of *Cyperus esculentus* were performed as previously reported[26,27].

**Data analysis**

Membrane stabilization, sperm characteristics (motility and viability) and phytoconstituents were presented as percentages. Other data were expressed as mean ± standard deviation (SD) of ten replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

**Results and discussion**

The traditional knowledge on the use of plants as aphrodisiacs has been embraced and is still finding relevance in providing succour and enhanced sexual performance in humans[28]. Plants known to rejuvenate sexual desire have been pharmacologically implicated to exert their influence on the hypothalamic-pituitary-testicular path of the animal system[21,29]. The results with respect to the effect of once daily dose administration of CEEAF on the motility, viability and counts of sperm cells showed a dose-dependent significant ($p < 0.05$) increase in these parameters when compared with the control over the period of investigation (Table 1). While the effect exhibited by the 30 mg/kg b.w. of CEEAF competed favourably with Powmax M, the best results with an overall 1.6 fold average improvement was observed with the 50 mg/kg b.w. of CEEAF over normal control for the three parameters (Table 1). During spermatogenesis or spermatozoa maturation, there is an increase in the number and quality of sperm cells production in the testes. In the present study, the significant and concentration-related increases in the motility, viability and total sperm count of the CEEAF-treated rats could be adduced to its spermatogenetic capability. This may be to either interfere with

Table 1 — Effects of ethyl acetate fraction of *Cyperus esculentus* on sperm motility, viability and counts in male rats

| | | | Fraction (mg/kg body weight) | | |
|---|---|---|---|---|---|
| Parameters | Control | PowMaxM | 15 | 30 | 50 |
| Motility (%) | 46.92 | 67.58 | 54.32 | 68.21 | 80.19 |
| Viability (%) | 42.60 | 58.49 | 49.31 | 59.31 | 67.39 |
| Count (Mio/mL) | $49.26 \pm 2.05^a$ | $60.11 \pm 3.01^b$ | $50.39 \pm 3.00^c$ | $62.31 \pm 2.92^b$ | $75.16 \pm 3.76^d$ |

Values with different superscript across the same row for each parameter are significantly ($p < 0.05$) different.

spermatogenetic process in the seminiferous tubules, aid epididymal functions or enhance testosterone actions on both hypothalamic release factor and anterior pituitary secretion of gonadotropins[30]. Specifically, the observed increase in the sperm motility across the treatment groups relative to control might be due to an alteration in the microenvironment in the cauda epididymis, which must have facilitated a synergistic action on the spermatozoa of the animals due to androgen-stimulatory potential of CEEAF. In addition, the effects elicited by the fraction may also be suggestive of its influence on spermatozoa maturation process which might have contributed to the increase in the total sperm count of the animals. These observations are in consonant with earlier findings[30,31], where improved sperm motility and quality were attributed to treatments with plant-derived formulations.

Following a 4-week administration of CEEAF (especially at 30 and 50 mg/kg b.w. doses), the relative testicular-body weight of the animals increased appreciably when compared with the rats placed on sterile placebo. The observed effects were significant ($p < 0.05$) and consistent with that of the reference drug (Table 2). While an increase in organ-body weight ratio may either depict inflammation or increased secretory ability of the organ, a reduction could be suggestive of cellular constriction. Thus, the observed increase in the relative testes-body weight ratio in the fractions-treated rats may be ascribed to increased secretory activity of the testes which is closely corroborated by the corresponding increases in the testicular levels of protein, cholesterol, glycogen and hormones (Tables 3 & 4) in this study. Such facilitated secretory potential of the testes following administration of CEEAF may further support its androgenic activity in rats and is consistent with the findings of Olaolu *et al*.[32] that linked progressive increase in testes-body weight ratio to administration of *Cissampelos mucronata* in male Wistar rats.

Protein, cholesterol and glycogen are important macromolecules to both spermatogenesis and subsequent maturation of sperm cells. While the lower doses of CEEAF competed with the reference drug, the most prominent effects were elicited by 50 mg/kg b.w. of CEEAF on all these parameters (Table 3). The dose-dependently increased testicular protein in the CEEAF-treated animals could possibly be due to stimulatory action of testosterone[33]. This pattern of moderation in protein concentration might aid sperm maturation which is crucial to androgenic tendency in animals and also buttress the aphrodisiac potential of CEEAF. Similarly, the increase in testicular cholesterol in the fraction-treated rats may suggest stimulation of steroidogenesis, thereby leading to increased androgen concentration[34] and probable androgenic potential of CEEAF. Glycogen is an important constituent of spermatogonia and Sertoli cells where it furnishes seminiferous tubular cells with optimum energy for uninterrupted germination, maturation and transportation of sperm cells[35]. The remarkably increased testicular glycogen concentration in the CEEAF-treated rats in this study could be adduced to either phosphorylase activation or its stimulatory influence on other metabolic enzymes linked with both spermatogenesis and androgenesis[36] and as such lending credence to the aphrodisiac tendency of the fraction. Hormones (testosterone, LH and FSH) have been closely associated with androgenicity and spermatogenesis[37], and treatments having modulatory influence on their concentrations could modify sexual behaviour and performance. In the gonads and under the influence of hypothalamic-pituitary axis of the brain, LH exerts stimulatory effect on testosterone by binding to receptors on Leydig cells. This consequently results in enhanced testosterone biosynthesis which is noteworthy in the present study (Table 4) and might be an indication that ethyl acetate fraction of *Cyperus esculentus* has stimulatory effect on the hypothalamic-pituitary portion of the brain of male

Table 2 — Effect of ethyl acetate fraction of *Cyperus esculentus* on the relative testes-body weight of male rats

| | | | Fraction mg/kg body weight | | |
|---|---|---|---|---|---|
| Parameters | Control | PowMaxM | 15 | 30 | 50 |
| IBW (g) | 227.09±6.19 | 220.25±5.60 | 238.65±6.31 | 241.09±7.32 | 258.75±5.54 |
| FBW (g) | 295.43±7.05 | 289.06±6.25 | 300.93±6.95 | 312.11±5.68 | 332.05±6.92 |
| TW (g) | 0.98±0.01[a] | 1.39±0.09[b] | 0.99±0.03[a] | 1.38±0.03[b] | 1.49±0.06[b] |
| RTW (%) | 0.33±0.02[a] | 0.48±0.01[b] | 0.33±0.01[a] | 0.44±0.02[b] | 0.45±0.03[b] |

Values with different superscript across the same row for each parameter are significantly ($p < 0.05$) different. IBW= Initial body weight, FBW= Final body weight, TW= Testes weight, RTW= Relative testes weight

Table 3 — Effect of ethyl acetate fraction of *Cyperus esculentus* on the testicular protein, cholesterol and glycogen levels of male rats

| Treatments | Protein (mg/mL) | Cholesterol (mmol/L) | Glycogen (mg/100 mg glucose) |
|---|---|---|---|
| Control | 215.36±4.63[a] | 0.92±0.05[a] | 2.05±0.15[a] |
| PowMaxM treated | 249.01±5.25[b] | 1.69±0.01[b] | 3.19±0.27[b] |
| 15 mg/kg CEEAFT | 245.62±5.31[b] | 1.76±0.09[b] | 2.92±0.11[b] |
| 30 mg/kg CEEAFT | 255.09±4.60[b] | 1.76±0.06[b] | 3.16±0.08[b] |
| 50 mg/kg CEEAFT | 296.15±3.68[c] | 1.99±0.02[c] | 3.99±0.10[c] |

Values with different superscript along the same column for each parameter are significantly ($p < 0.05$) different. CEEAFT= *C. esculentus* ethyl acetate fraction treated

Table 4 — Effect of ethyl acetate fraction of *Cyperus esculentus* on the testicular hormones of the rats

| | | | Fraction (mg/kg body weight) | | |
|---|---|---|---|---|---|
| Hormones | Control | PowMaxM | 15 | 30 | 50 |
| FSH (IU/L) | 1.99±0.05[a] | 2.99±0.03[b] | 2.75±0.08[b] | 3.62±0.09[c] | 3.99±0.01[c] |
| LH (IU/L) | 3.52±0.25[a] | 4.24±0.31[b] | 3.99±0.46[a] | 4.38±0.19[b] | 4.92±0.32[b] |
| Testosterone(nmol/L) | 26.05±1.62[a] | 40.01±3.50[b] | 42.61±3.61[b] | 49.23±3.01[b] | 50.62±3.92[c] |

Values with different superscript across the same row for each parameter are significantly ($p < 0.05$) different. FSH= follicle stimulating hormone, LH= luteinizing hormone

rats. Testosterone is required for the growth and development of male reproductive organs[32] and in association with FSH, acts on the seminiferous tubules to initiate and maintain spermatogenesis[22]. In the present investigation, the extract had a direct effect on the testes that resulted to increased testosterone production and could therefore be tempting to suggest that treatment with CEEAF may be responsible for the observed significant masculine and sexual competences of the male animals. In addition to invigorative orgasm and sensational ejaculation, testosterone supplementation has also been reported to improve sexual function and libido[38]. Generally, the effects elicited by CEEAF on the hormonal regulations in this study is an indication that *Cyperus esculentus* could support normal functioning of the Sertoli cells which will concomitantly enhance spermatogenesis, sperm cell maturation and androgenicity.

Compared with the standard drug (ibuprofen), the results of membrane stabilization revealed that the fraction was dose-specifically potent on rat erythrocyte, significantly protecting it against hypotonic solution and heat induced lyses (Table 5). The results of the phytochemicals screening of the fraction indicated that it is rich in saponin, flavonoids and alkaloids (Table 6) and these metabolites might be responsible for the elicited activities in this study. For instance, as an aphrodisiac agent, alkaloids have been implicated to either induce vasodilation of blood vessels through nitric oxide production that consequently favours penile erection or stimulate steroidogenesis in the testes of animals[8]. In addition to being antioxidative, flavonoids and saponins have also been studied to stimulate testosterone biosynthesis via synergistic influence on luteinizing hormones, which in turn results in male sexual vigour and competency[39]. The presence of these phytonutrients in the fraction may also be responsible for its significant membrane stabilization effect. This was evidently shown in this study with the fraction at 2 mg/mL conferring membrane stabilization potentials of 89.25 % and 85.71 % on rat erythrocytes against hypotonic solution and heat-induced injuries, respectively. This could either be ascribed to the capability of the fraction to bind firmly to the RBC membranes with attendant prevention of inimical blitz or its potential in promoting dispersion by mutual repulsion of the charges as being involved in RBC hemolysis. Sabiu & Ashafa[25] also gave similar submission while testing membrane stabilization effect of crude ethanolic extract of *E. obliqua*. Thus, the fact that ethyl acetate fraction of CE is phytoconstituent-rich might be another justifiable reason for the enhanced sexual performance displayed by the male rats in this study and may be ascribed to their antioxidative, membrane stabilization, and aphrodisiac potentials.

Table 5 — Membrane stabilization effect of *Cyperus esculentus* ethyl acetate fraction

| Treatment | Conc (mg/mL) | Hypotonic hemolysis | | Heat hemolysis | | |
|---|---|---|---|---|---|---|
| | | Absorbance | % inhibition | $A_H$ | $A_U$ | % inhibition |
| Control | 0.00 | 0.93±0.01[a] | - | 0.82±0.01[a] | 0.55±0.01[a] | - |
| | 0.25 | 0.75±0.02[b] | 19.35 | 0.25±0.02[b] | 0.18±0.02[b] | 10.94 |
| Fraction | 0.50 | 0.59±0.02[c] | 36.56 | 0.49±0.02[c] | 0.30±0.02[c] | 36.54 |
| | 1.00 | 0.55±0.01[c] | 40.86 | 0.52±0.01[c] | 0.35±0.01[c] | 36.17 |
| | 1.50 | 0.34±0.02[d] | 63.44 | 0.61±0.02[d] | 0.46±0.02[d] | 41.67 |
| | 2.00 | 0.10±0.03[e] | 89.25 | 0.79±0.03[e] | 0.61±0.03[e] | 85.71 |
| Ibuprofen | 0.10 | 0.11±0.02[e] | 88.17 | 0.75±0.02[e] | 0.54±0.02[e] | 75.00 |

[abcde]Values with different superscripts along the same row for each parameter are significantly different ($p < 0.05$). $A_H$ = Absorbance of heated solution, $A_U$ = Absorbance of unheated solution.

Table 6 — Phytochemical composition of ethyl acetate fraction of *Cyperus esculentus*

| Phytonutrients | % composition |
|---|---|
| Glycosides | 1.20 |
| Tannins | 2.53 |
| Alkaloids | 9.67 |
| Flavonoids | 19.98 |
| Saponins | 25.21 |

## Conclusion

Consequent upon the significant androgenic and membrane stabilization activities of ethyl acetate fraction of *Cyperus esculentus* in this study, it could be inferred that the plant is endowed with antioxidant and phytotherapeutic constituents. This has not only justified the rationale behind the use of the plant as aphrodisiac but also enrich the traditional information on the pharmacological significance of *Cyperus esculentus* in the traditional system of medicine.

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