**In vitro and in vivo Effects of Khaya grandifoliola C. DC. (Meliaceae) Leaf on Erectile Dysfunction in Male Rats**

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**Abstract**

**Background:** *Khaya grandifoliola* C. DC. (Meliaceae) is exploited as a popular antimalarial herbal medicine in Nigeria, externally in treating skin diseases, and to enhance libido.

**Objectives:** This study evaluates the aphrodisiac potential of *K. grandifoliola* leaf in male Wistar rats.

**Materials and methods:** Aphrodisiac property was determined by oral administration of graded doses: 100, 250 and 1000 mg/kg of crude methanol (MeOH) extract of *K. grandifoliola* leaf, and 100 and 200 mg/kg each of aqueous (AQ) and dichloromethane (DCM) fractions to male rats using standard procedure. Sildenafil citrate and distilled water served as positive and negative controls, respectively. Sexual behavioural parameters like mounting, intromission and ejaculatory frequencies and latencies were recorded on day 7. Anti-lipid peroxidation effect and serum testosterone concentrations were also monitored.

**Results:** From the sexual behavioural study, MeOH extract of the leaf was active and significantly increased mount and ejaculatory frequencies, and decreased mount and ejaculatory latencies in a dose dependent manner, but had no effect on intromission latency and intromission frequency. Both AQ and DCM fractions were more active than extract, comparable to Sildenafil citrate, but DCM fraction was comparatively more active. Testosterone concentrations were also increased dose-dependently by all tested agents, but DCM fraction was the most active and comparable to Sildenafil citrate. Only DCM fraction significantly inhibited lipid peroxidation but was not comparable to Vitamin E.

**Conclusion:** From all indications, MeOH extract and fractions of *K. grandifoliola* leaf which increased serum testosterone and sexual behavioural indices has potential for improving sexual dysfunction in males, and the more active DCM fraction qualifies for further phytochemical investigation to isolate active compounds. These findings therefore justify the acclaimed local use of *K. grandifoliola* leaf in treating male infertility.

**Keywords:** *Khaya grandifoliola*, aphrodisiac activity, *In vivo* model, *In vitro* model, Anti-lipid peroxidation effect

**INTRODUCTION**

Erectile dysfunction is characterized by delayed ejaculation, frigidity, impotence anorgasmia, sexual aversion, dyspareunia and premature ejaculation (Valentin et al., 2020). This increasing sexual dysfunction is more prevalent in women (25 - 63%) than in men (10 - 50%) according to global estimates (Valentin et al., 2020). Based on a ten-year review from 1999 to 2009 by Valentin et al. (2020), a prevalence of 0 to 3% for orgasmic dysfunctions, 0 to 5% for erectile dysfunctions and 0 to 3% for dysfunctions of male hypoactive sexual desire have been documented in the male population. Global reviews on plant aphrodisiacs include those of Mali (Togola et al., 2020), DR Congo (Valentin et al., 2020), and...
2020), sub-Saharan Africa (Ajao et al. 2019) and over 700 plants listed by Sin et al. (2021).

Importance of aphrodisiac research in Nigeria is evidenced in the publication of Ajao et al. (2019) which ranked Nigeria third with 28 plants in aphrodisiac inventories in sub-Saharan Africa. Scientific reports on the Nigerian aphrodisiac plants have been documented by Ajao et al. (2019). The Literature abound on high prevalence of sexual dysfunction according to the community survey in Ogbomoso, South west Nigeria involving male population aged 30 to 80 years (58.9%) (Oyelade et al., 2016), Ibadan hospital survey on 18-70 years old male population (55.1%) (Adebusoye et al., 2012) and in over 40 years old males in Turkey (69.2%) (Akkus et al., 2002). Ethnobotanical surveys of aphrodisiac plants in Nigeria have been published for Akwa Ibom state with 31 plants in six families (Erhabor et al., 2013) and Bauchi Local Government Area with 10 plants (Sabo et al., 2017). Elsewhere in Africa, Ajao et al. (2019) reported 209 traditional aphrodisiac plants, but only 48 plants out of the 77 plants scientifically investigated (including Gloriosa superba, Macuna pruriens, Sphenocentrum jollyanum and Morella serrata) were found to elicit activity by increasing testosterone levels in rats, index of libido, sperm motility and prolonged ejaculation latency. Recently, Gbolade et al. (2022) reported Khaya grandifoliola stem bark as an enhancer of serum testosterone level and sexual behavioural indices in male rats with activity residing in dichloromethane fraction.

African mahogany, Khaya grandifoliola C. DC. (Meliaceae), is a large tree growing in West Africa, Sudan and Uganda, up to 40 m in height, with a straight trunk and dark brown bark reaching a girth exceeding 1 m (Yusuf et al., 2021). Apart from being exploited for its timber for commercial purposes, the plant is valuable in indigenous traditional medicine in West Africa to treat illnesses like malaria fever, lumbago, cough, rheumatism, stomach complaints, gastric pains, and as remedy against worm infestation (Yusuf et al., 2021). It is also used traditionally for erectile dysfunction in south western Nigeria (personal communication). The phytochemistry, pharmacological and toxicological properties of K. grandifoliola was recently reviewed by Yusuf et al. (2021).

Although literature is lacking on information on aphrodisiac potential of K. grandifoliola, a related species, K. senegalensis is listed in the Malian ethnobotanical survey of traditional remedies for erectile dysfunction (Togola et al., 2020). We therefore investigated K. grandifoliola using both in vitro and in vivo models in the search for potent plant-based aphrodisiacs for the management of increasing male sexual dysfunction.

### METHODOLOGY

**Plant material and extraction**

Leaves of K. grandifoliola were collected from Okeigbo in Ondo state, Nigeria in December 2019 and authenticated (voucher no. FHI 107644) at FRIN herbarium, Ibadan, Nigeria. They were cut into small pieces, air-dried for 7 days and mechanically powdered to obtain a coarse powder which was exhaustively extracted (1 kg) in a Soxhlet apparatus with analar grade methanol. Crude extract was concentrated on a water bath (40°C) to give a residue which was fractionated with dichloromethane (DCM) to yield DCM and aqueous (AQ) fractions. Dried crude extract and fractions were weighed and refrigerated (4°C) until required.

**Phytochemical screening**

Basic phytochemical screening was carried out on the crude methanol extract of the plant according to Sengar et al. (2009) and the presence of secondary metabolites recorded.

**Animals**

Wister rats of both sexes (male 150-300 g and females 120-160 g), and 48 mice (20-25 g) were used. Male and female rats were kept separately for two weeks in cages in the animal house of Department of Pharmacology, Igbinedion University Okada at 28-35°C under artificial (12h light/12h dark) lighting system, and maintained on grower mash feed (Bendel Foods and Flower Meal, Edo state, Nigeria) and water ad libitum.

**Procedure for oestrus cycle of female rats**

Following the procedure of Yakubu and Akanji (2011), acclimatized female Wister rats were weighed on the 5th day, and then artificially brought into oestrus phase (heat) by oral administration of Tween 80 solutions of estradiol benzoate (10 µg/100 g) daily for 48h and progesterone (0.5 mg/100 g) 4h prior to physical study of sexual behaviour. The experiment was conducted between 19:00 and 22:00 h at the Postgraduate Research Laboratory of the College of Pharmacy.
**Determination of male rat sexual behaviour**

This experiment, performed as described by Yakubu and Akanji (2011), was initiated after the approval of the Animal Ethics Committee of College of Pharmacy, Igbinedion University Okada.

Initial weights of male Wister rats were determined and they were divided into five groups of five animals each. Positive control group A received Sildenafil citrate (100 mg/kg) orally, negative control group B received distilled water (1 mL/kg), while groups C - E received 250, 500 and 1000 mg/kg of crude MeOH extract, respectively for 7 days. The experiment was repeated separately with 100 mg/kg and 200 mg/kg of AQ and DCM fractions each. The Guide for the care and use of laboratory animals (DHHS, 1985) was followed in this protocol.

**Measurement of sexual parameters**

Receptive female animals were introduced into the cages of male animals in the ratio 1 female to 1 male thrice daily for 4 days after a 30 min adaptation period. The observation for mating behaviour (proceptive and precopulatory) commenced immediately and continued for one hour, and on days 1, 3 and 7 afterward (Yakubu and Akanji, 2011). Occurrence of events and phases of mating was recorded using digital video recorder (Canon VIXIA HF10, camcorder), and frequency determined from video transcriptions. Various male sexual behaviour indices were assessed according to Tang et al. (2017) as follows: Mount frequency (MF, the number of mounts at a specified period of time without intromission from the time of introduction of the female), Intromission frequency (IF, the number of intromissions from the time of introduction of the female until ejaculation), Ejaculation frequency (EF, the number of ejaculations from the time of introduction of the female rats to the male within a given time frame), Mount latency (ML, the time interval between the introduction of the female and the first mount by the male), Intromission latency (IL, the time interval from the time of introduction of the female to the first intromission by the male), Ejaculation Latency (EL, the time interval between the first intromission and ejaculation). This is usually characterized by pelvic thrusting and springing dismount.

**RESULTS AND DISCUSSION**

A low yield of MeOH extract (9.5%) was obtained in this study (Table 1), while metabolites detected include alkaloids, saponins, steroids flavonoids. Anthraquinone glycosides, cardiac glycosides, terpenoids and phenolic compounds. With sexual behaviour frequency index, all tested agents gave dose-dependent increases in mount frequency (MF) and ejaculation frequency (EF) (Table 2).

**Estimation of testosterone concentration**

The animals were anaesthetized in a jar containing cotton wool soaked in diethyl ether. When rats became unconscious, their neck region was quickly cleared of fur and skin to expose their internal jugular veins (Yakubu and Akanji, 2011). The veins were slightly displaced (to prevent contamination of the blood with interstitial fluid) after which they were cut sharply with a sterile blade. The rats were then held head downwards, allowed to bleed into clean, dry centrifuge tubes. Blood samples were allowed to clot for 10 min at room temperature and subsequently centrifuged at 22 × g for 10 min with Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England). The sera were aspirated with Pasteur pipette and used for the determination of testosterone concentration within 12 h of preparation (Yakubu and Akanji, 2011).

**Anti-lipid peroxidation assay (TBARS)**

A modified thiobarbituric acid-reactive species (TBARS) assay as described by (Adedokun et al., 2021) was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid rich medium. Egg homogenate (0.5 mL of 10% v/v) and 0.1 mL of leaf methanolic extract were added to a test tube and made up to 1 mL with distilled water. 0.005 mL of FeSO₄ (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% sodium dodecyl sulphate (SDS) and 0.5 mL 20% trichloroacetic acid (TCA) were added and the resulting mixture was cooked at 95°C for 60 minutes. After cooling, 5.0 mL of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Incubation of lipid peroxidation (%) by the extract was calculated according to:

\[
\text{% Inhibition} = \frac{(\text{Control Absorbance} - \text{Control Extract}) \times 100}{\text{Control Absorbance}}
\]
Table 1: Profile of *Khaya grandifoliola* and yield of extract and fractions

<table>
<thead>
<tr>
<th>Plant</th>
<th>Voucher number</th>
<th>Morphological part</th>
<th>Aspect</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Khaya grandifoliola</em></td>
<td>FHI 107644</td>
<td>Leaf</td>
<td>Tree</td>
<td>Okeigbo, Ondo state</td>
</tr>
</tbody>
</table>

Yield

<table>
<thead>
<tr>
<th>Crude methanol extract</th>
<th>Aqueous fraction*</th>
<th>Dichloromethane fraction*</th>
</tr>
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<tbody>
<tr>
<td>9.5%</td>
<td>28.68%</td>
<td>32.28%</td>
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</tbody>
</table>

*Relative to weight of crude extract used

Table 2: Effect of crude extract and fractions of *Khaya grandifoliola* leaf on sexual behaviour in rats after 7 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mount latency (sec) (ML)</th>
<th>Intromission latency (sec) (IL)</th>
<th>Ejaculation latency (sec) (EL)</th>
<th>Mount frequency (Mount/hr) (MF)</th>
<th>Intromission frequency (IF)</th>
<th>Ejaculation frequency (EF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (Distilled water)</td>
<td>34.75±9.72</td>
<td>11.00±0.82</td>
<td>47.00±7.38</td>
<td>5.50±0.29</td>
<td>4.75±0.47</td>
<td>n. d.</td>
</tr>
<tr>
<td>Positive control (Sildenafil citrate)</td>
<td>22.50±3.57</td>
<td>19.00±1.29</td>
<td>33.00±4.45</td>
<td>16.75±1.11</td>
<td>87.00±7.68</td>
<td>2.50±0.29</td>
</tr>
<tr>
<td>Crude MeOH extract, 100 mg/kg</td>
<td>28.25±7.73</td>
<td>10.75±1.11</td>
<td>61.00±7.01</td>
<td>19.00±5.82</td>
<td>122.30±10.09</td>
<td>1.25±0.25</td>
</tr>
<tr>
<td>Crude MeOH extract, 250 mg/kg</td>
<td>28.25±5.45</td>
<td>12.00±0.82</td>
<td>40.25±8.31</td>
<td>11.00±0.71</td>
<td>114.50±22.89</td>
<td>2.00±0.48</td>
</tr>
<tr>
<td>Crude MeOH extract, 1000 mg/kg</td>
<td>27.50±3.12</td>
<td>16.50±1.32</td>
<td>43.50±4.33</td>
<td>16.50±1.85</td>
<td>96.50±4.99</td>
<td>2.00±0.48</td>
</tr>
<tr>
<td>AQ fraction 100 mg/kg</td>
<td>43.00±8.89</td>
<td>1.25±0.63</td>
<td>55.00±8.39</td>
<td>1.50±0.65</td>
<td>19.25±11.28</td>
<td>1.50±0.28</td>
</tr>
<tr>
<td>AQ fraction 200 mg/kg</td>
<td>27.25±5.12</td>
<td>3.00±1.08</td>
<td>40.75±6.97</td>
<td>2.25±1.75</td>
<td>16.75±8.47</td>
<td>1.75±0.25</td>
</tr>
<tr>
<td>DCM fraction 100 mg/kg</td>
<td>31.25±5.11</td>
<td>13.00±0.91</td>
<td>46.75±3.49</td>
<td>13.00±1.16</td>
<td>84.00±6.75</td>
<td>0.50±0.99</td>
</tr>
<tr>
<td>DCM fraction 200 mg/kg</td>
<td>28.00±4.71</td>
<td>17.25±1.49</td>
<td>37.75±5.54</td>
<td>16.00±0.71</td>
<td>88.00±4.56</td>
<td>0.75±0.02</td>
</tr>
</tbody>
</table>

Values above are mean of six replicates. n=6 (±SEM). Values with superscripts * indicate significant difference at P<0.05 when compared to negative control, using ordinary One–way analysis (ANOVA). n. d: not determined

DCM fraction was more active (13-16 mounts/h) than AQ fraction, and equipotent with extract at 250-1000 mg/kg in MF activity. Only extract and DCM fractions (16-16.50 mounts/h) at their highest doses gave comparable MF results with Sildenafil citrate. In addition to AQ fraction being more active (1.50-1.75 ejaculations) than DCM fraction (0.5-0.75 ejaculations) in EF activity, it was also comparable with extract at 250-1000 mg/kg and Sildenafil citrate. Only DCM fraction gave slight dose-dependent increase (84-88 intromissions) in IF which is significantly (P < 0.5) greater than AQ fraction (19.25-16.75 intromissions), and comparable with extract at 1000 mg/kg and Sildenafil citrate (87 intromissions). MeOH extract was active and exhibited dose-dependent decrease in mount latency at tested doses of 100 -1000 mg/kg (Table 3). The two fractions gave comparable reduction in ML (AQ 43 - 27.25 sec, DCM 32.25 - 28 sec) and were also comparable to Sildenafil citrate at higher dose (200 mg/kg). This is in tandem with the publications on *Massularia acuminata* aqueous extract (Yakubu and Akanji, 2011), *Allium* species, *Garcinia cola* and *Cola acuminata* aqueous extracts (Nwafor et al., 2020) and *K. grandifoliola* stem bark methanol extract and fractions (Gbolade et
All three tested agents were inactive as regards intromission latency as they produced dose-dependent increases rather than decrease (Yakubu et al., 2005; Nwafor et al., 2020). On ejaculatory latency, dose-dependent decreases with extract and the two fractions were recorded. Active fractions were comparable to standard drug in EL activity at 200 mg/kg. These observations are in agreement with the inverse relationship of ML to sexual motivation proposed by some workers (Yakubu and Akanji, 2011; Nwafor et al., 2020). The observed decrease in the mount latency in this study, might imply stimulation of sexual motivation and arousability (Yakubu and Akanji, 2011) leading to enhanced sexual appetitive behaviour in the male rats. With reference to all sexual indices, both fractions and extract were equipotent in ML and EL activities, but extract was active more in EF activity and equipotent with DCM fraction in MF activity. Consequently, only DCM fraction would qualify for further phytochemical investigation to isolate bioactive constituents.

Behaviour of extract and fractions of K. grandifoliola leaf agreed with those of other workers (Yakubu and Akanji, 2011; Tang et al., 2017; Nwafor et al., 2020; Gbolade et al., 2022), and is a pointer to its aphrodisiac potential. Yakubu and Akanji (2011) postulated that the increase in IF as observed in this study, may be due to activation of the mechanism of penile erection. Other mechanisms of aphrodisiac action of plant extracts have been published (Oyelowo et al., 2012; Nurudeen et al., 2015; Sabiu et al., 2016). MF and IF are useful indices of vigour, libido and potency, and the increases observed by both fractions in this study, suggest (Yakubu and Akanji, 2011; Erhabor and Idu, 2017.) enhanced libido probably due to elevated anterior pituitary hormonal and serum testosterone levels, which in turn stimulated dopamine receptor synthesis and sexual behaviour. It may therefore be logical to attribute these behaviours to flavonoid and or saponin constituents of the plant since they have been reported (Tang et al., 2017) to alter androgen levels. In, summary, and with reference to all indices of sexual behaviour, MeOH extract and DCM fraction followed the traditional increases in MF and EF only, and decreases in EL and ML, and thus have clearly demonstrated potential as aphrodisiac agents.

In this present study, dose-dependent increases in testosterone levels were recorded with MeOH extract and fractions (Figure 1), suggestive of enhanced sexual desire by male rats. DCM fraction (5.60-13.35 nM/L) was more potent than MeOH extract (1.89-2.90 nM/L) when tested at similar doses (100 mg/kg and 200 mg/kg. It was also twice as active (5.60 nM/L) as AQ fraction at 100 mg/kg, and at 200 mg/kg (13.35 nM/L) in enhancing serum levels of this male hormone.

Active DCM fraction will therefore be a candidate for further phytochemical work to identify bioactive compounds. Elevated serum testosterone concentrations in sexually impaired animals has been suggested as one of the mechanisms of aphrodisiac action (Yakubu et al., 2005; Yakubu and Akanji, 2011; Erhabor and Idu, 2017; Gbolade et al., 2022). Sexual enhancement has also been attributed to dehydroepiandrosterone (DHEA), a major circulating steroid in the plasma and a common precursor for both androgen and estrogen synthesis, that is subsequently converted to testosterone and its metabolites (Tang et al., 2017).

Figure 1. Effect of methanol extract and fractions of K. grandifoliola leaf on blood testosterone level.
Values above are mean of six replicates. n=6 (±SEM). Values with superscripts * indicate significant difference at P<0.05 when compared to negative control using ordinary One-way analysis (ANOVA).

From Figure 2, apart from Vitamin E, only DCM fraction gave concentration-dependent increase in inhibition of lipid peroxidation from 400 – 1000 µg/mL. It showed 2-fold activity (16.70-17.81%) of AQ fraction (7.95-8.98%) at 800 – 1000 µg/mL. Although MeOH extract was the most active tested agent (29.90-34.60% inhibition), however, it was not concentration-dependent. Order of anti-lipid peroxidation activity was: extract > DCM fraction > AQ fraction. In addition, all tested agents were incomparable to standard drug in inhibition of lipid peroxidation.

Figure 2. TBARS activity (% inhibition) of Khaya grandifoliola leaf methanol extract
Values above are mean of six replicates. n=6 (±SEM). Values with superscripts * indicate significant difference at P<0.05 when compared to positive control using ordinary One-way analysis (ANOVA).

CONCLUSION
MeOH extract and fractions of K. grandifoliola leaf, investigated for the first time, promoted sexual behaviour in male rat. Aphrodisiac activity was believed to reside in both fractions for all mount and ejaculatory sexual indices except intromission values, and particularly in DCM fraction which was the most potent agent in enhancing testosterone level. Overall, the more potent DCM fraction in this study may be subjected to further phytochemical work to discover bioactive compounds. K. grandifoliola leaf reported herein for the first time for its aphrodisiac potential, is an update on the compendium of Nigerian medicinal plants with aphrodisiac potential.

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