COMPARATIVE PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* (MORINGACEAE) EXTRACTS AND REPRODUCTIVE EFFECTS OF THE AQUEOUS LEAF, SEED AND BARK EXTRACTS IN RATS

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ABSTRACT

*Moringa oleifera* (moringa) is widely consumed for its several nutritional and medicinal benefits. This study analysed phytochemical constituents (alkaloids, flavonoids, tannins, anthraquinones, cardiac glycosides, carbohydrates, and saponins) of crude aqueous and ethanol extracts of leaf, seed, flower, bark and root of the plant collected from the southern part of Nigeria. The study also evaluated the effect of 21 days administration of different concentrations of the aqueous seed or bark extract (100, 200 and 400 mg/kg bwt) on sperm parameters using Wistar albino rats. The study additionally investigated the outcome of pregnancy when the aqueous leaf extract (200 mg/kg bwt) was administered from GD1 or GD7 till delivery in Wistar rats. All phytochemical compounds tested positive only in the flower (aqueous and ethanol), leaf (ethanol) and bark (ethanol) extracts. Overall, the ranking of phytoconstituents in the plant parts was: *flower > seed > leaf > root > bark*. There was reduction (p<0.05) of sperm motility in aqueous seed or bark extracts treated rats, but no changes occurred in their sperm count and morphology when compared with control. Maternal body weight was not affected by the leaf extract, neither did it alter gestation period, number of pups delivered or birth weight of pups, but successful pregnancy rate was reduced. In conclusion, the flower may contain more phytochemical compounds than other...
parts, whereas the bark the least. In addition, moringa seed or bark may reduce sperm motility without affecting count and morphology, while the leaf may induce pregnancy loss in rats.

**KEYWORDS:** Abortifacient, moringa, phytochemical, pregnancy, sperm parameters

**INTRODUCTION**

*Moringa oleifera* is a fast growing, drought-resistant and multipurpose tree. It is widely cultivated in various tropical and sub-tropical areas, but is indigenous to the southern foothills of the Himalayas in north-western India, which remains its largest producers. *Moringa oleifera* belongs to the family Moringaceae, and is the most widely cultivated among 13 species in the genus Moringa. *Moringa oleifera* is popularly called “The Miracle Tree” because of its numerous health benefits. Some of its other common names include, Drum Stick, Horseradish Tree, Ben-oil Tree or Benzoil Tree, Mothers’ Best Friend, Radish Tree, Never Die Tree, The Tree of Life, The Miracle Tree, and West Indian Ben Tree.\(^{[1-3]}\)

The various parts of the plant—root, bark, stem, leaf, fruit (pod) and seed have been reported to be very useful for nutritional, industrial or medicinal purposes.\(^{[4,5]}\) For medicinal purposes, moringa is used to treat numerous ailments in ethnomedicine.\(^{[2,5]}\) Furthermore, the plant has been established to possess a wide range of pharmacological activities and most of its numerous activities and health benefits have been attributed to its phytochemical constituents.\(^{[6-8]}\) Phytochemical analysis have shown that the plant is rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, carbohydrates, as well as potent antioxidant compounds like β-carotene, vitamin C, and flavonoids.\(^{[9-11]}\)

The reproductive system is concerned with fertilization of the egg by spermatozoa and subsequent development of the embryo. The testis and pregnancy period are generally sensitive to chemical agents,\(^{[12,13]}\) however this caution is not observed in most rural areas as herbal products are consumed frequently by many including pregnant women, relying on the local belief that natural products are not harmful. Although, some studies have reported the influence of moringa on reproductive organs, the results are not exhaustive as not all parts of the plant have been sufficiently investigated.\(^{[14-16]}\) More so, the geographical location where the plant is harvested may affect the result that is obtained.

The objective of this study was to analyse and compare the phytochemical composition of the aqueous and ethanol extracts of different parts of moringa obtained in Port Harcourt, a city in
the southern part of Nigeria. The study was equally aimed at evaluating the effect of administering the aqueous seed or bark extract on sperm parameters as well as studying the influence of the aqueous leaf extract on pregnancy in rats.

**MATERIALS AND METHODS**

**Plant samples preparations**

Fresh *Moringa oleifera* leaves, seeds, flowers, barks and roots were collected from a moringa tree in a garden at Borikiri, Port-Harcourt, Nigeria and identified by a botanist, Dr Okeke Chimezie of the Department of Plant Science and Biotechnology of University of Port-Harcourt. They were assigned voucher number (UPH/P/60) and samples were deposited in our University’s herbarium. The plant materials were air-dried for 7 days and ground to form powder which were extracted with distilled water or ethanol to obtain the aqueous and ethanol extracts, respectively.

Briefly, to obtain the aqueous extracts, the samples were macerated in distilled water in a ratio of 1:4 for 24 h during which appropriate pressure was exerted for proper maceration and filtered using Whatman filter paper. The filtrates of the different parts were concentrated using steam bath at 40°C to obtain the dry leaf, seed, flower, bark and root extracts and their percentage yields were calculated. To obtain the ethanol extracts, the samples were extracted by soxlet extraction using ethanol at a temperature of 78°C (boiling point of ethanol). Extracts obtained were transferred into crucible plates and heated in steam bath at 40°C to obtain dry extracts. Percentage yield of the extracts (leaf, seed, flower, bark and root) were also calculated.

**Phytochemical screening of extracts**

Phytochemical screening was done on all the extracts for the presence of alkaloids, anthraquinones, flavonoids, tannins, cardiac glycosides, carbohydrates and saponins using standard methods.[17]

**Experimental protocol**

Non-pregnant female Wistar albino rats (200 g body weight) and male Wistar albino rats (300 g body weight) were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt. They were kept in a well ventilated room, fed with standard rodent diet and given water *ad libitum* under natural lighting condition and room temperature
of 26±4°C. Animal ethics and proper handling methods were strictly adhered to,\textsuperscript{[18]} and all experimental procedures were approved by our University’s Ethics Committee.

**Study of moringa extract influence on sperm parameters**

Thirty-five male rats were grouped into 7 (n=5 per group) and gavaged orally with aqueous seed extract (100, 200 or 400 mg/kg bwt), aqueous bark extract (100, 200 or 400 mg/kg bwt) or vehicle (distilled water plus dimethyl sulphoxide) daily for 21 days. Animals were anesthetized with diethyl ether and sacrificed by cervical dislocation. The testis was isolated and sperm fluid was squeezed out from the caudal epididymis and sperm motility, count and morphology were quantified using standard methods as previously described.\textsuperscript{[19]}

**Study of moringa extract influence on pregnancy indices**

Male and female rats were mated (1:1) and pregnant rats (confirmed by presence of vaginal plug by sperm) were separated for the study. The pregnant rats (30) were divided into 3 equal groups. The first group was administered extract (200 mg/kg) from gestation day (GD)1 till delivery. The second group was treated with extract (200 mg/kg) from GD7 till delivery. The third group (control group) received vehicle from GD1 till delivery. Body weights of animals during pregnancy were monitored thrice and the gestation length in each group was noted. After delivery, the litter size, and birth weight of pups were obtained, as well as number of rats that had successful deliveries.

**Statistical analysis**

Data were analysed using GraphPad Prism 5 software and represented as mean±Standard Error Mean (SEM). Comparison of values between experimental and control groups was performed by one way analysis of Variance (ANOVA) where applicable and values were considered significant if p<0.05.

**RESULTS**

**Yield and phytochemical constituents of moringa extracts**

The percentage yields of the aqueous and ethanol extracts of the leaf, seed, flower, bark and root were different and is shown in Tables 1 and 2. The yields followed a rank order of, flower>bark>leaf or root>seed for the aqueous extracts; and flower>bark>seed>root>leaf for the ethanol extracts.
Seven phytochemicals were analysed - alkaloid, flavonoid, tannin, anthraquinone, cardiac glycoside, carbohydrate and saponin. For the aqueous extracts, all the phytochemicals were detected in the flower; the seed and bark tested positive for five compounds, while the leaf and root showed presence of only four compounds. Tannin, cardiac glycoside and saponin were present in all the parts, while flavonoid was absent in all except the flower (Table 1). For the ethanol extracts, all the phytochemicals were present in the leaf, seed, and flower. Alkaloid, tannin and cardiac glycoside were absent in the bark, while the root showed absence of alkaloid (Table 2).

Table 1: Yield and phytochemical constituents of aqueous extracts of different parts of *Moringa oleifera*.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Yield (%)</th>
<th>Phytochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaloid</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>Leaf</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>Seed</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Flower</td>
<td>39</td>
<td>+</td>
</tr>
<tr>
<td>Bark</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Root</td>
<td>14</td>
<td>-</td>
</tr>
</tbody>
</table>

+ (present), - (absent)

Anthraq: Anthraquinone; Carbo: Carbohydrate

Table 2: Yield and phytochemical constituents of ethanol extracts of different parts of *Moringa oleifera*.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Yield (%)</th>
<th>Phytochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaloid</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>Leaf</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Seed</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>Flower</td>
<td>23</td>
<td>+</td>
</tr>
<tr>
<td>Bark</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Root</td>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

+ (present), - (absent)

Anthraq: Anthraquinone; Carbo: Carbohydrate

Effects of aqueous seed and bark extracts on sperm parameters

Sperm motility was reduced (p<0.05) in rats that were administered seed or bark extract when compared to the control group (Figures 1a and 2a). But sperm count and morphology values
obtained in the rats were not altered compared to control (Figures 1b and 1c). The result was similar in the rats that were treated with bark extract (Figures 2b and 2c).

![Graphs showing sperm motility and count](image)

Figure 1: Administration of aqueous seed *Moringa oleifera* seed extract for 21 days reduced (a) sperm motility without affecting (b) sperm count and (c) morphology in rats.

Data are expressed as mean±SEM, n=5 per group

* Significant at p<0.05.
Figure 2: Administration of aqueous bark *Moringa oleifera* bark extract for 21 days reduced (a) sperm motility without affecting (b) sperm count and (c) morphology in rats.

Data are expressed as mean±SEM, n=5 per group

* Significant at p<0.05, ** Significant at p<0.01, *** Significant at p<0.001.

**Effect of aqueous leaf extract on pregnancy indices**

Initial body weight of control rats increased significantly (p<0.05) at GD20. Similar observation was made in rats that received extract (Figure 3). Gestation periods of rats that received extract on GD1 (23.67±0.53 days) or GD7 (24.11±0.87 days) were not altered when compared to the gestation length of 24.27±0.79 days that was obtained in control rats (Figure 4). Further, 9 out of the 10 (90%) rats in control group gave birth at the end of the study, whereas 60% gave birth in the group that received extract on GD1, and 70% gave birth in the
group that received extract on GD7. The litter size delivered by rats in control and the two extract treated groups were comparable and did not statistically vary from each other (Table 3). Similarly, the birth weight of pups delivered by extract treated rats were not different compared to control (Table 3).

![Graph showing body weight changes during pregnancy](image.png)

**Figure 3:** *Moringa oleifera* leaf extract consumption during pregnancy does not affect body weight in Wistar rats.

Data are expressed as mean±SEM, n=10 per group

*Significant at p<0.05, compared to GD1 [Observed in control and Extract (GD1) treated groups].

**Significant at p<0.01, compared to GD1 [Observed in Extract (GD7) treated group].

GD: Gestation Day

Extract (GD1): Extract (200 mg/kg) administered from GD1 to end of pregnancy.

Extract (GD7): Extract (200 mg/kg) administered from GD7 to end of pregnancy.
Figure 4: Administration of *Moringa oleifera* leaf extract on GD1 or GD7 during pregnancy does not alter gestation length in Wistar rats.

GD: Gestation Day

Extract (GD1): Extract (200 mg/kg) administered from GD1 to end of pregnancy.

Extract (GD7): Extract (200 mg/kg) administered from GD7 to end of pregnancy.

**Table 3**: The influence of *Moringa oleifera* leaf extract on some pregnancy indices in Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. that gave birth</th>
<th>Litter size</th>
<th>Birth weight of pups (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>8.36±0.64</td>
<td>5.48±0.06</td>
</tr>
<tr>
<td>Extract (GD1)</td>
<td>6</td>
<td>7.90±0.18</td>
<td>5.48±0.11</td>
</tr>
<tr>
<td>Extract (GD7)</td>
<td>7</td>
<td>7.78±0.70</td>
<td>5.17±0.24</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM, n=10 per group

GD: Gestation Day

Extract (GD1): Extract (200 mg/kg) administered from GD1 to end of pregnancy.

Extract (GD7): Extract (200 mg/kg) administered from GD7 to end of pregnancy.

**DISCUSSION**

*Moringa oleifera* is a valuable medicinal plant which has also been demonstrated to be pharmacologically very potent. Different parts of the plant contain a profile of important phytochemical constituents that are mostly responsible for its biological actions.\(^6,20\) Part of this study analysed five different parts of moringa plant (leaf, seed, flower, bark and root)
obtained from a southern city in Nigeria with respect to the phytochemical contents of their extracts.

The results revealed that aqueous and ethanol extracts of most of the parts contained alkaloid, flavonoid, tannin, anthraquinone, cardiac glycoside, carbohydrate and saponin which were tested in this study. For the aqueous extracts, the flower contained all the 7 phytochemicals that were evaluated; while the rest did not contain at least two, including flavonoid. For the ethanol extracts, the leaf, seed and flower contained all 7 phytochemicals, the bark was positive for all except alkaloid, whereas the root did not contain alkaloid, tannin and cardiac glycoside. Additionally, the seven phytochemical compounds were present only in the flower (aqueous and ethanol), leaf (ethanol), and bark (ethanol) extracts. This makes ethanol a better solvent capable of extracting organic and inorganic materials from the plant. Overall, the ranking of phytoconstituents in the plant parts was flower>seed>leaf>root>bark. This did not correspond exactly to the ranking of the percentage yields- flower>bark>seed>root or leaf.

Furthermore, the result obtained after three weeks treatment with aqueous seed or bark extract of moringa in male rats showed that the seed or bark extract would inhibit motility of spermatozoa. This is not the same with sperm count or sperm morphology as neither the seed nor bark extract caused alteration of the two sperm indices. Thus, the extract may possibly not affect sperm quantity or sperm production processes but could affect sperm quality which is essential for fertility.\textsuperscript{[21,22]}

In the female reproductive study, the results showed that the aqueous leaf extract did not interfere with body weight gain during pregnancy, suggesting that it may not affect maternal health. Maternal body weight is a vital physiological indicator of maternal health and may reflect growth or developmental status of the fetus.\textsuperscript{[23]} This was confirmed in the birth weight of pups that were delivered by extract treated mothers. Agents that cause low birth weights are known to be harmful to pregnancy and induce negative health consequences to the offspring.\textsuperscript{[23,24]} Gestation duration and number of pups delivered are other important pregnancy indices, prolongation or shortening of the length of gestation is not healthy for the fetus. The results indicated that none of these was affected by the extract. Further, it was observed that pregnancy progressed successfully to the point of delivery in only 60% of the rats that received extract on GD1, suggesting that the extract may induce loss of pregnancy when consumed during early pregnancy in rats. Earlier, moringa leaf from geographical
locations outside Nigeria had been reported to have abortive effect after administering 175 mg/kg for 10 days from beginning of conception in rats.\textsuperscript{16} In India, the bark of moringa had been reported to be used traditionally as an abortifacient agent.\textsuperscript{16} Other parts of moringa like the root and bark have equally been demonstrated to induce abortifacient or anti-infertility effect in female animals.\textsuperscript{14,15} Some of the phytoconstituents of moringa like alkaloids and saponins possess anti-fertility potentials and may contribute to the results that were obtained in the present study.\textsuperscript{25-27} This raises issues with the overall safety of the plant during pregnancy, even though the plant may not affect the other pregnancy indices discussed above. This is particularly important as it is a welcome practice in most traditional settings for the use of herbal preparations to treat infertility or enhance fertility.\textsuperscript{28}

In conclusion, results obtained from this study showed that the flower of moringa may contain higher phytoconstituents than other parts, while the bark contained the least. Also, the leaf may not prevent maternal weight gain or affect pregnancy indices like gestation length, and number and birth weight of pups delivered, but it may induce loss of pregnancy in rats. In addition, the seed or bark may reduce sperm motility.

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CONFLICTS OF INTEREST
None.

REFERENCES