

Ethnopharmacological Assessment of *Cyathea manniana* Hook: Antifertility Activity and Acute Toxicity in Swiss Albino Rats

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Short Report

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Abstract

Ethnopharmacological Relevance: *Cyathea manniana* Hook (Amharic: Sheshino) is one of the traditionally used medicinal plants and a multipurpose tree fern that belongs to the family Cyatheaceae. The leaves of this plant are traditionally utilized for their antifertility and antibacterial activities by the Shekicho and Kafficho people, who are native to the southwestern part of Ethiopia.

Aim of the Study: To investigate the abortifacient and acute toxic effects of the hydro-ethanolic extract of *Cyathea manniana* leaves in Swiss albino rats.

Methods: Dried leaf powder of the plant was extracted using 80% ethanol in water, yielding a crude plant extract after evaporation. The extract was screened for major secondary metabolites using standard phytochemical screening techniques. Fertilization was induced by mating mature female rats with males overnight in a metallic mating cage. The antifertility effects of the crude extract were examined at doses of 300 mg/kg and 600 mg/kg. Rats were anesthetized with diethyl ether for laparotomic assessment of the uterus, ovaries, and corpora lutea. A one-way ANOVA was used to compare the mean number of corpora lutea between control and experimental groups.

Results: The plant extract was relatively safe up to a dose of 500 mg/kg, with no deaths or apparent organ toxicity observed. Compared to the controls, both the 300 mg/kg and 600 mg/kg doses of the extract exhibited 80% and 100% anti-implantation activity, respectively, as well as 80% abortive activity at both doses. The extract tested positive for major metabolites, including alkaloids, flavonoids, saponins, steroids, and tannins.

Conclusions: The findings of this study support the traditional claims regarding the use of *Cyathea manniana* for antifertility purposes. Further studies should be conducted to assess the activity of the fractionated extract, elucidate potential compounds responsible for its activity, and explore the molecular mechanisms underlying its antifertility effects.

Clinical Trial Number: Not applicable.

Background

General Background

The use of herbal preparations for abortion is widespread, especially in Sub-Saharan Africa where these products are readily available. Several animal studies have demonstrated that these herbal products can inhibit fertilization through one of three mechanisms: blast cytotoxicity, anti-implantation, or inducing uterine contraction. It is hypothesized that they achieve anti-implantation effects by antagonizing hormones necessary for maintaining pregnancy, such as progesterone, similar to the mechanism of the well-known drug used for pregnancy termination, mifepristone. Additionally, in some cases, these effects have been found to be dose-dependent. ^[1,2,3]

An abortifacient effect refers to the ability to induce abortion, defined as the dislodging of the implanted blastocyst from the endometrium before it develops into a viable fetus, leading to the termination of pregnancy. The time frame for legally defining fetal viability may vary across countries, depending on their ability to sustain the fetus. For instance, in the Ethiopian scenario, it is at 28 weeks of gestation. Globally, 25% of pregnancies were terminated by abortion during 2010–14. According to Clark A. and Amanda R., 10-20% of clinically recognized pregnancies will end in early pregnancy loss [4,5,6,7].

The use of herbal medicines for treating common ailments is as old as human history. Even in the era of modern medicine, up to 80% of the world population relies on herbal medicines to maintain their health and well-being. This reliance may be particularly high in developing countries like Ethiopia, where access to modern medicine is limited, and cultural and religious beliefs influence the preference for traditional medicine. In addition to their common uses for preventing and treating medical conditions, plant-based preparations have been employed for managing gynecologic and obstetric conditions, such as preventing pregnancy, managing infertility, and inducing abortion [8,9].

The acute toxic effects of herbal medicines used for abortion are a serious concern due to their lack of regulation and unknown constituents. These medicines can lead to organ toxicity, allergic reactions, gastrointestinal disturbances, and hormonal disruption, which may have long-term implications for reproductive health. Interaction with other medications is also possible. Women should exercise caution, seek guidance from healthcare providers, and be aware of potential risks before using herbal preparations for abortion. Healthcare professionals must remain vigilant for signs of toxicity and provide appropriate medical care to mitigate adverse outcomes [6,8,11].

Botanical Background of *Cyanthea manniana* Hook.

***Cyanthea manniana* Hook. f.** is a multipurpose tree-fern belongs to the family of Cyatheaceae (37), which could grow up to 10 meters height and 15 cm trunk width. The plant usually grows in the wet shady forest area and the river valleys of Wet Kolla and Weyna Dega agroclimatic zones (**Fig 1**). It is abundant in a way from Tepi to Mizan district, Southwest of Ethiopia [7].

The stem part of the plant is used for house construction, fencing, and firewood [9]. It was reported that the leaves of the plant are employed for the treatment of sexually transmitted diseases by Kafficho people [5,10,11]. In an ethnobotanical study commissioned by Mizan-Tepi University, the leave extract of *C. manniana* is commonly used to induce abortion by the local Shekicho people and it was similarly reported the use of the plant for abortion in West African communities [8].

Due to the cultural stigma surrounding abortion in Ethiopia, women facing unintended pregnancies often avoid seeking healthcare services for such procedures. This is further exacerbated by limited access to healthcare facilities in remote regions. As a result, women may turn to locally available herbs as an alternative method for terminating pregnancies. However, it is important to consider that the consumption of these herbs, whether intentionally or unintentionally, can have adverse effects on

women's health. In light of these circumstances, the present study aimed to investigate the traditional claim of *C.manniana* as an abortifacient and evaluate its acute toxicity profile [43,44.45]

Methods and Materials

Study Site, Period and Study Design

The research was carried out at the Biomedical and Pharmaceutical Laboratory and Animal Research Facility of Mizan Tepi University College of Health Sciences. It was a pre-clinical, confirmatory, prenatal developmental toxicity, a randomized control experimental study done in 2021.

Used Chemicals for extraction and content identification

80% methanol, Ferric chloride, sulphuric acid, lead acetate, Ammonium hydroxide, acetic acid anhydride, normal saline, distilled water, diethyl ether, and chloroform. ^[12] .

Plant material Identification and Collection

The fresh leaves of *Cyathea manniana hook* were collected from forestry regions of Sheka zone, Southwest Ethiopia. Authentication of the plant specimen was done by a taxonomist, with a sample deposited at the Herbarium of the College of Health Science, Mizan-Tepi University (MTU) (Specimen voucher No: 036/MTU/PHARM) for future reference. ^[13,14] .

Preparation of crude extract

The leaves were cleaned from any extraneous materials and dried under a shade at room temperature. The dried leaves were crushed into a fine powder. The powder was macerated with a hydro-ethanolic solvent (80% ethanol in water). Initially, 310g of plant powder was macerated in a chamber with an adequate amount of 80% ethanol and covered with a lid. The maceration continued for three days with occasional stirring, then filtered using a muslin cloth followed by a Grade 1 Whatman filter paper. The filtrate was heated at 80°C temperature in a water bath till the solvent was completely evaporated, yielding a crude extract. ^[15,16] Sample photographs showing the extraction within the Pharmacology laboratory, Mizan–Tepi University is attached (**Fig 2**)

Phytochemical screenings.

The extract was tested for the presence of common plant metabolites using standard techniques pertinent to different classes of compounds. The methods employed are briefly described below.

The extract was tested for tannins and flavonoids using ferric chloride and lead acetate test, respectively. Approximately 1g of extract (0.5g for each) was dissolved in 100mL of distilled water and poured via a muslin filter. For each respective test, a small amount of filtrate was added into separate test tubes and treated with a few drops of 5% of ferric chloride solution and 10% of lead acetate solution. Froth test was used to detect saponins; by dissolving 150mg of extract in 20ml of distilled water and shaken vigorously for the development of foam. [15,17]

Mayer's test was used for the detection of alkaloids by dissolving 0.5g of extract in 5mL of dilute H_2SO_4 with steam bathing, followed by filtering. The filtrate was then treated with a few drops of Mayer's reagent. Tests against steroids and terpenoids were done using Libermann Burchard's and Salkowski's methods, respectively. Initially, for each test, 0.5g of extract was mixed with 2mL of chloroform and then filtered. In the case of steroids, the filtrate was treated with 2mL of acetic anhydride and 2 mL concentrated H_2SO_4 , whilst only concentrated H_2SO_4 was added for the terpenoids test. Finally, Borntrager's test was used for the detection of anthraquinones. Accordingly, the extract was mixed with chloroform, shaken, and filtered. An equal amount of ammonium hydroxide was then added to the filtrate [17,18]

Experimental Animals

A breed of 15 Swiss albino rats aged between 1 and 2.5 months were got from Ethiopian Public Health Institutes, Addis Ababa, Ethiopia. Enough number of rats were then bred at the laboratory of Mizan-Tepi University, Ethiopia, under recommended conditions. The breeding was taken place in well-ventilated stainless-steel cages under natural light and dark schedules. They were fed with rodent standard pellets and water ad-libitum. Before the actual experiment, rats were acclimatized to laboratory conditions for one week to alleviate any non-specific stress. The procedures for this study were conducted following the guidelines for the ethical treatment of research animals and were granted approval by the Departmental Ethics Committee [17,18,19]. Sample photographs showing the Experimental Swiss albino rats in their cage at Animal Research Facility of Mizan Tepi University College of Health Sciences is attached (**Fig 3**).

Assessment of Acute toxicity

The acute toxicity study of the extract was carried out using matured female Swiss albino rats weighing between 150-250g. The toxicity was tested at six different oral doses (300, 500, 1000, 2000, 2500 and 5000mg/Kg) with six treatment groups of five rats each. Each dose was dissolved in distilled water and administered orally. There was a control group (five rats) that received distilled water. The acute toxicity study was conducted according to the OECD guideline 423 [18]. Animals were fasted overnight before dosing to enhance absorption. They were continuously monitored during the first four hours of dosing and every 12 hours then after for 14 days. Any signs of behavioural and physiologic changes, and mortality were recorded. We closely observed any toxicity signs; such as piloerection, lethargy, loss of

appetite, dizziness, hypoactivity, and convulsion^[24]. At the end of the second week, the final weight of each rat was recorded, and they were humanly sacrificed with a high dose (3500mg/kg) of anaesthetic diethyl ether. In liver and kidney, signs of acute organ damages were assessed using a gross pathologic feature (congestion and haemorrhage) and microscopic change (fatty degeneration and occasional cellular necrosis).^[20,21]

Assessment of Abortifacient effect

Female and male matured rats were mated as described by Choudhary using metallic breeding cages with meshes^[11]. Pregnancy was considered when detecting sperm from the vaginal smear was taken as day one of gestation^[46,47].

Briefly, for anti-implantation activity, two doses of plant extract (300 and 600mg/Kg per day) were administered starting from the third day of gestation for three consecutive days in three different groups of five albino rats (five control, five 300 mg/kg receiving and five 600/kg receiving). The control group (five rats) received 1.0 mL of distilled water for three days. On day 9, rats were anesthetized with diethyl ether (3500 mg/Kg) and laparotomies to count implantation, adsorbing sites, and corpora lutea^[43,44].

For the abortion study, 15 pregnant rats on day 10 of gestation were randomly divided into three groups. Five rats as control and ten experimental group (five rats in 300mg/kg taking group and other five rats grouped in 600mg/kg taking group). The 10 experimental group rats given their respective doses from day 10 to 12 of gestation and laparotomies on day 13. The control group given 1.0 mL normal saline. Following laparotomy, the number of viable and non-viable fetus, adsorbing sites and corpora lutea were counted. Complete placental detachment, percent of fetus viability and vaginal bleeding were used to assess abortifacient effect and the presence of vaginal bleeding was followed since day 10^[23].

Statistical analysis

Continuous variables were summarized with means and standard deviations while using count for categorical variables. A one-way ANOVA was fitted to compare the difference in means live fetus between groups at a significant level $p < 0.05$. Dunnett's post-hoc test was conducted for multigroup comparisons to determine their level of significance.

Results

Extraction Yield

The extraction yield was estimated using the following formula. 310 grams powder macerated

- Weight of evaporating dish =26.3 grams
- Total weight = 96.8 grams

$$yeild = \frac{total\ weight - weight\ of\ evaporating\ dish}{weight\ of\ plant\ material} \times 100 = \frac{70.5g}{310g} \times 100 = 22.75\%$$

Phytochemicals

We identified a diverse group of phytochemicals that could be implicated in the biological activities of the plant under investigation (**Table 1**).

Acute toxicity of the extract

Single oral doses of *C. manniana* Hook extract did not cause death up to a maximum recommended toxicity threshold (5000mg/Kg). Some symptoms of behavioral changes were observed at a dose of ≥ 2500 mg/Kg. The symptoms of behavioral changes got worse with increasing dose, such symptoms, however, gradually waned out and disappeared after a week of administration. (**Table 2**). The experimental and control groups gain weight during the two weeks of observation. The difference in weight gain in all groups was not significant ($P > 0.05$) (**see Table 3**). Gross pathology feature acute organ damage were not observed in liver, heart and kidney of the experimental group similar to the control group. There was no lesion on those organs that potentially attributed to the toxicity of the extract.

Abortifacient effect of the extract

Anti-implantation was observed in 80% and 100% of the treatment group at 300 and 600 mg/Kg, respectively. (**Table 4**) However, there were no significant association between MNCL and the hydro-alcoholic extract of *C. manniana*.

In the assessment of abortifacient effect, the mean numbers of corpora lutea were 1.34 ± 3.92 ($p = 0.004$) and 1.79 ± 4.65 ($p = 0.003$) at a dose of 300mg/kg and 600mg/kg, respectively. Moreover, the mean number of viable foetuses at day 13 was 3.17 ± 2.17 ($P = 0.007$) and 3.0 ± 2.23 ($P = 0.004$) at 300 and 600 mg/Kg, respectively. These is much lower than that of the control group and indicates the abortifacient effect. Evaluation of the extract for its abortifacient activity starting from the 10th day of gestation also showed an abortive action in pregnant rats in for out of five (80%) and for out of four (80%) rats, at 300 and 600 mg/Kg, respectively. Generally, abortifacient effect was not observed in the control group including vaginal bleeding. (**Table 5**).

Discussion

In this experimental study, we examined the antifertility activity of *C. manniana* leaves extract. On phytochemical screenings, the extract was positive for alkaloids, flavonoids, saponins, tannins, and steroids. A study conducted in Congo similarly reports flavonoids from the species ^[28]. In contrary to our study, alkaloids, tannins, and steroids were not reported in a study conducted by Koto-te-Nyiwa Ngbolua and his colloquies ^[29]. Such variation might be due to the different geographical area/ soil conditions, the weather condition, time of plant collection, part of the plant, and the chemicals used for the extraction. These researchers have done the screening of the phytochemicals from the whole part of the plant.

With a single oral dose of up to 5000mg/kg, the hydro-alcoholic extract of *C. manniana* revealed no animal death. The administration of extracts to animals over 5000mg/kg has not been carried out with a view to animal welfare ^[17]. Loss of appetite, hypoactivity, piloerection, tiredness, and dizziness are some of the behavioural changes observed starting from 2500mg/Kg. Symptoms, on the other hand, disappeared after the first week of observation. As a result, our findings imply that the extract's oral LD50 (50 percent lethal dose of the experimental animal) is greater than 5000mg/Kg. As a result, the plant extract is regarded safe for oral consumption. The hydro-alcoholic extract of *C. manniana* hindered implantation in pregnant rats, which was dose dependent. Oral administration of *C. manniana* leaf extracts to pregnant rats on days 3–5 of gestation inhibited blastocyst implantation and resulted in a significant reduction of corpora lutea. Authors Hijazi and Salhab conducted a similar study on *Artemisia monosperma* extracts for implantation and mid-term abortive effect on female pregnant rats that found similar results ^[30]. Likewise, an oral administration of a hydro-alcoholic leaves extract of *Alstonia scholaris* Linn found results consistent with our study ^[24].

C. manniana extracts were also found to have strong abortifacient properties. At both doses, female pregnant rats showed a dose-dependent loss of implants and an increased number of resorptions. The findings of this study support the plant's historic reputation as an abortifacient, as do many other species in the Cyatheaceae family ^[31,32,33]. Several plants were reported for their reputation of antifertility activity ^[25, 30, 31, 32, 33,34, 35, 36] and were experimentally investigated for those claims ^[3, 4,16, 22, 24, 37, 39 ,40].

Furthermore, the antifertility effect could be attributed to the effects of *C. manniana* metabolites on hormonal balance, blood progesterone levels, estrogenic activities, and ovarian and uterine development ^[3,4,24, 37, 38, 41]. Even though it is difficult to pinpoint which metabolites have anti-implantation and abortifacient properties, phenolic compounds such flavonoids have been discovered in numerous plants with antifertility properties ^[24,32,33,40]. And also, the exact mechanism(s) of this observed anti-fertility activity of those metabolites remains unclear ^[3,4].

Conclusions

In conclusion, the hydro-ethanolic extract of *Cyathea manniana* leaves demonstrated significant anti-implantation and abortifacient activities in Swiss Albino rats without causing apparent organ toxicity up to a dose of 500mg/Kg. The study substantiates the traditional use of *Cyathea manniana* in Southwestern Ethiopia by Shekicho and Kafficho people for antifertility purposes. These findings contribute to the growing body of ethnopharmacological knowledge and may offer insights for the development of novel contraceptives or reproductive health interventions. However, caution should be exercised in the clinical application of *Cyathea manniana* extracts until further studies validate its safety and efficacy in pregnant wommens. Moreover, we recommend the scientific community study on the drug pharmacokinetic and pharmacodynamics on animal subjects as our laboratory setup is not equipped to do such study to step up to clinical study.

Abbreviations

C. manniana	<i>Cyathea manniana</i>
ANOVA	Analysis of variance
mg/Kg	Milligram per kilogram
ml	Millilitre
g	Gram
FeCl₃	Iron chloride
H₂SO₄	Sulphuric acid
OECD	Organization for Economic Cooperation and Development

Declaration

Ethics approval and consent to participate

The research was carried out with the approval of the research ethics review committee of Mizan-Tepi University's College of Health Sciences, with the letter-number MTU/CHS/2006. The animals in this investigation were kept safe from any painful or frightening situations. Animals were handled according to protocol and were sedated for any uncomfortable procedures. (WHO, 1993; WHO, 2000; OECD, 2001)

Consent to publish

Permission to publish and disseminate the results of the study was granted by the Mizan-Tepi University, Research directorate office.

Availability of Data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

All the necessary equipment and chemicals for this study were fully covered by the research directorate office of Mizan-Tepi University, Ethiopia

Authors' Contributions

NED : is involved in the conception and design of the study, drafting and revising the article, and final approval of the version to be submitted.

MAK: is involved in the conception and design of the study, drafting and revising the article, and final approval of the version to be submitted.

ABT: involved in the conception and design of the study, drafting and revising of the article, and final approval of the version to be submitted.

TLD: involved in the conception and design of the study, drafting and revising of the article, and final approval of the version to be submitted.

HTA: involved in the conception and design of the study, drafting and revising of the article, and final approval

ATK: Involved in the conception and design of the study, drafting and revising of the article, and final approval of the version to be submitted.

HAS: Involved in the conception and design of the study, drafting and revising of the article, and final approval of the version to be submitted.

KGY: Involved in the conception and design of the study, drafting and revising of the article, and final approval of the version to be submitted.

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Tables

Table 1. The Phytochemicals identified from C. manniana extracts

Secondary metabolites	80% Ethanol extract
Alkaloids	Positive
Flavonoids	Positive
Saponins	Positive
Steroids	Positive
Tannins (hydrolyzable)	Positive
Anthraquinones	Negative
Terpenoids	Negative

Table 2. Acute toxic effects of C. manniana Hook leaf extract

The dose of <i>C. manniana</i> Extract (mg/ kg)	Death/experimented	Symptoms of toxicity	
		During 1 st week of study	During 2 nd week of study
300	0/5	None	None
500	0/5	None	None
1000	0/5	None	None
2000	0/5	None	None
2500	0/5	Loss of appetite, Hypo-activity	None
5000	0/5	Hypo-activity, lethargy, loss of appetite	None
Control	0/5	None	None

Table 3. Mean body weight of rats treated with C. manniana extract as compared to the controls during the two weeks observation period (expressed as mean $\pm$ SDE, n = 5)

Group	Dose (mg/kg)	Initial mean body weight (gm)	Mean Body weight at the end of week 1(gm)	Mean Bodyweight on day 14 (gm)
I	300	197.4± 21(0.63)	210.33 ± 18.9(0.66)	222.33±19.07(0.56)
II	500	185.33± 17.3(0.55)	195.4 ± 21.24(0.36)	204.07±20(0.62)
III	1000	202.42±7.64(0.7)	212 ± 8(0.79)	220.8 ± 13.95(0.80)
IV	2000	193.9±6.34(0.57)	201.32 ± 8.65(0.31)	212.55±7.22(0.17)
V	2500	198 ± 6.6(0.59)	204.5 ± 6.83(0.60)	208.55±5.62(0.64)
VI	5000	202 ± 8.55(0.35)	208.1 ± 9.17(0.46)	216.67±9.54(0.53)
VII	Control(vehicle)	185.5 ± 7.17	194.85 ± 7.41	206.5 ± 7.84

Table 4. Anti-implantation effects of hydro-alcoholic extract of C. manniana on female pregnant rats.

Parameters	Control	Extract (mg/kg)	
		300	600
Number of pregnant	5	5	5
Anti implantation activity	Nil	80%	100%
Mean Number of corpora lutea	14±4.26	12±1.12(p> 0.05)	13±3.46(p>0.05)

Table 5. Abortifacient effects of hydro-alcoholic extract of C. manniana on female pregnant rats (expressed as mean ± SDE, n = 4)

Parameters	Control	Extract (mg/kg)	
		300	600
Number of pregnant	5	4	4
Vaginal Bleeding	N0	25%	30%
Mean Number of corpora lutea	13±4.26	12±3.33(p>0.05)	11±2.65(p>0.05)
Mean number of Viable fetuses	12±1.34	3.17 ±2.17 (P < 0.007)	3.0 ±2.23 (P < 0.004)
Abortifacient effect	0	80%	80%

Figures

Figure 1



Figure 1

Photograph of Leaves of C. manniana. Taken by Research team, Sheka Forest, Ethiopia, 2018.

Figure 2



Figure 2

Sample photographs showing the extraction within the Pharmacology laboratory, Mizan–Tepi University, Ethiopia, 2021.

Figure 3



Figure 3

Sample photographs showing the Experimental Swiss albino rats in their cage at Animal Research Facility of Mizan Tepi University College of Health Sciences Mizan–Tepi University, Ethiopia, 2021