

Original article

Effects of *Silene apetala* Aqueous Extract on Offspring Sex Ratio in Mice: A Pilot Ethnobotanical and Experimental Study

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Abstract

Silene apetala (locally known as "Barrama") is a plant traditionally used by Libyan women to influence the sex of their offspring; however, scientific validation of this practice remains undocumented. This study was designed to investigate the ethnobotanical basis, phytochemical composition, and potential biological effects of the whole plant aqueous extract of *S. apetala* on offspring sex ratio in a mouse model. An ethnobotanical questionnaire was administered to 66 Libyan women to document traditional knowledge. Whole plants (including roots and aerial parts) were collected, authenticated, and an aqueous decoction was prepared. Qualitative phytochemical screening was performed using standard protocols. In the experimental study, eight female Swiss albino mice were evaluated using a paired design. Offspring were counted and sexed in two phases: Phase 1 (Control/Pre-treatment) and Phase 2 (Treatment with 100 mg/kg BW extract). Statistical analysis was performed using Fisher's Exact Test. The survey indicated that 93.9% of participants reported positive outcomes regarding male offspring. Phytochemical screening revealed strong concentrations of flavonoids (+++) and moderate levels of cardiac glycosides (++) , while saponins were notably absent (-). In the experimental study, the proportion of male offspring increased from 45.8% (22/48) in the control phase to 55.8% (29/52) in the treatment phase. Although a 10% increase was observed, the difference was not statistically significant ($p = 0.2399$). These findings provide preliminary scientific support for the ethnobotanical use of *S. apetala*. The observed trend suggests that the plant's bioactive flavonoids and steroidal glycosides may influence reproductive outcomes, though larger-scale studies are needed to achieve statistical significance.

Keywords. *Silene apetala*, Sex Ratio, Ethnobotany, Phytochemicals, Reproductive Biology.

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Introduction

Traditional medicinal plants continue to be a cornerstone of healthcare in North Africa and many developing regions globally. The World Health Organization estimates that a significant portion of the population, up to 80%, relies on these traditional remedies for their primary healthcare needs [1]. This knowledge is often passed down verbally through generations, particularly among women who address fertility and reproductive concerns [2].

In Libya, a plant known as *Silene apetala* L. (locally called 'Barrama') is traditionally used by women with the intention of influencing offspring sex, particularly favoring male children. This ethnobotanical knowledge was documented through structured interviews in the present study (see Methods, Section 2.1). Despite this ongoing traditional practice, there have been no experimental studies to scientifically investigate the biological mechanisms behind its purported effects.

The *Silene* genus is quite diverse, encompassing over 500 species found worldwide. In Libya alone, taxonomic studies have identified 23 *Silene* species, including *S. apetala*, which grows in both Mediterranean coastal areas and inland regions [3]. Various *Silene* species are known to produce diverse bioactive secondary metabolites, including saponins, flavonoids, phytoecdysteroids, and phenolic compounds [4,5,6]. These compounds have been shown to exhibit a range of biological activities, including hormone-modulating, antioxidant, and reproductive effects in various experimental models [7,8]. More specifically, phytoecdysteroids-steroid-like compounds abundant in several *Silene* species [9] have been shown to exert hormone-like effects in mammals, including modulation of stress response and potential influence on reproductive

physiology [8,10]. Additionally, flavonoids, particularly isoflavones and other polyphenolic phytoestrogens, can bind to estrogen receptors and modulate reproductive hormone signaling in mammals [11,12].

While mammalian sex is fundamentally determined by chromosomes, maternal factors can play a role in influencing sex ratios. These factors include diet, hormonal status, and exposure to certain phytochemicals. They can impact sex ratios by affecting aspects like gamete quality, the timing of fertilization, or the early stages of embryonic development [13,14]. Experimental evidence in mammals has demonstrated that maternal condition and metabolic status can facultatively adjust offspring sex ratios, supporting the Trivers-Willard hypothesis [15].

Although some Libyan women use *S. apetala* and related *Silene* species that contain bioactive compounds, no experimental studies have assessed whether this plant can affect reproductive physiology or offspring sex ratios. Therefore, the present study investigates the ethnobotanical background, phytochemical profile, and potential reproductive effects of *S. apetala*, aiming both to document traditional knowledge and to expand scientific understanding of medicinal plants in reproductive health.

Materials and Methods

Ethnobotanical Survey

A structured ethnobotanical survey was conducted among 66 Libyan women to document traditional knowledge and use patterns. The survey employed a standardized questionnaire administered through face-to-face interviews and collected information on plant identification, preparation methods, and perceived efficacy.

Plant Material and Extract Preparation

Whole plants of *Silene apetala* (including roots, stems, leaves, and flowers) were collected from natural habitats in Tripoli during the flowering season. Authentication was performed at the Herbarium of the Faculty of Pharmacy, University of Tripoli. To simulate the traditional decoction method, 150 g of the dried whole plant powder was boiled in 1000 mL of distilled water for 30 minutes. During the boiling process, the volume was reduced through evaporation to a final concentrated volume of 250 mL. This decoction, representing a raw plant equivalent of 600 mg/mL (60% w/v), was filtered through Whatman No. 1 paper and stored at 4°C until use.

Phytochemical Screening

Qualitative phytochemical analysis was performed on both aqueous and alcoholic extracts to identify major secondary metabolites. Standard qualitative tests were performed to detect major classes of secondary metabolites (flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, and coumarins) according to established methods [16,17]. The intensity of each compound class was recorded as absent (-), weak (+), moderate (++), or strong (+++).

Experimental Animals

Eight adult female Swiss albino mice (25-30 g body weight, 8-10 weeks old) were obtained from the animal facility of the Faculty of Pharmacy, University of Tripoli. Animals were housed in standard polypropylene cages under controlled environmental conditions and acclimatized for one week prior to the start of the experiment. All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Experimental Design

A paired experimental design was employed in which each mouse served as its own control, thereby reducing inter-individual variability and increasing statistical power. The study was conducted in two phases:

Phase 1 (Pre-treatment/Control): Female mice were mated with proven fertile male mice (1:1 ratio) without any treatment. After confirmation of mating (presence of vaginal plug), females were separated and monitored throughout gestation. After parturition, offspring were counted and sexed based on anogenital distance (greater in males) and confirmed after maturation of the external genitalia.

Phase 2 (Treatment): After a recovery period of 4 weeks post-weaning, the same female mice were administered *S. apetala* aqueous extract orally using a calibrated dosing syringe. The dose was 100 mg/kg body weight, adjusted individually according to each mouse's weight. The extract was administered daily for 7 days prior to mating with the same male partners as in Phase 1, and administration continued until pregnancy was confirmed. After parturition, offspring were counted and sexed using the same methods.

Body weights of female mice were monitored weekly throughout both phases. General health status, behavior, and any adverse effects were observed daily.

Statistical Analysis

Offspring sex ratio data were analyzed using Fisher's Exact Test (two-tailed) to compare the proportion of male offspring between control and treatment groups. Statistical significance was set at $p < 0.05$.

Results

Ethnobotanical Survey Results

Of 66 women surveyed, 62 (93.9%) reported positive outcomes regarding male offspring after using *Silene apetala* (Barrama) (Table 1). Traditional preparation involved decoction of the whole plant, consumed daily starting before conception and continuing until pregnancy occurs.

Table 1: Ethnobotanical survey results of *Silene apetala* use among Libyan women

Parameter	Number	Percentage
Total respondents	66	100%
Reported positive outcomes (male offspring)	62	93.9%
Reported no effect	4	6.1%

Phytochemical Screening

Qualitative phytochemical analysis of *Silene apetala* extracts revealed a strong presence of flavonoids (+++) and a moderate presence of cardiac glycosides (++) . Tannins were weakly detected (+), whereas saponins, anthraquinones, and coumarins were absent (-) (Table 2).

Table 2: Phytochemical screening of *Silene apetala* extracts

Compound Class	Intensity
Flavonoids	+++
Cardiac glycosides	++
Tannins	+
Saponins	-
Anthraquinones	-
Coumarins	-

Legend: (+++) strong presence, (++) moderate presence, (+) weak presence, (-) absent

Animal Health and Behavior

All eight female mice tolerated the extract well throughout the study period. No adverse effects, behavioral changes, or mortality were observed during either phase of the experiment. Body weights remained stable with normal weight gain patterns during gestation. Maternal behavior, including nest building and pup care, appeared normal in both phases.

Reproductive Outcomes and Offspring Sex Ratio

All eight female mice successfully mated and produced offspring in both the pre-treatment (control) and treatment phases, demonstrating that the extract did not impair fertility or reproductive capacity at the dose used. In the pre-treatment phase, a total of 48 offspring were born, with 22 males (45.8%) and 26 females (54.2%). Following extract administration, 52 offspring were born, with 29 males (55.8%) and 23 females (44.2%). This represents an increase of 10 percentage points in the proportion of male offspring after treatment (Table 3, Figure 3). Individual variability was observed among the eight female mice, with some showing more pronounced changes in offspring sex ratio between the two phases than others.

Table 3: Offspring sex distribution before and after *S. apetala* extract treatment

Phase	Total Offspring	Male Offspring	Female Offspring	Male Proportion (%)
Pre-treatment (Control)	48	22	26	45.8
Post-treatment	52	29	23	55.8

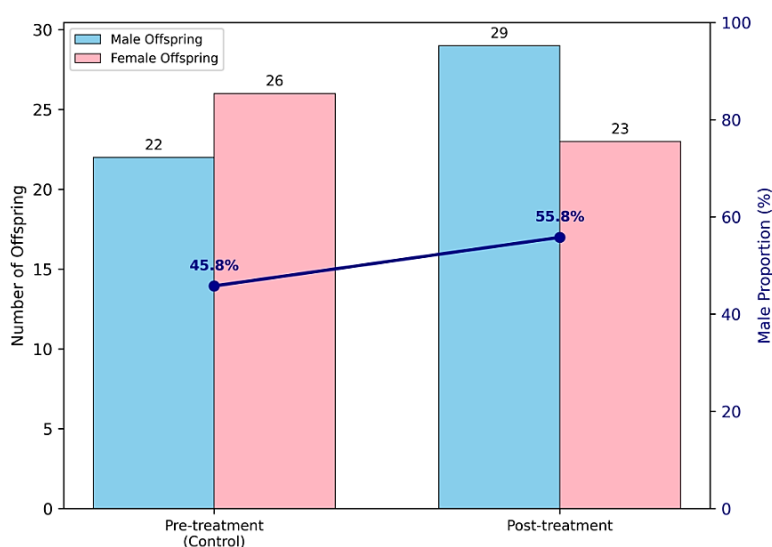


Figure 3: Offspring sex distribution before and after *S. apetala* extract treatment

Discussion

The use of the whole plant (including roots) in this study was based on traditional preparation practices rather than targeting a specific class of metabolites. Although roots in many *Silene* species are reported to contain bioactive compounds [6,18], saponins were not detected in the present analysis, which may reflect species-specific variation or limitations of qualitative screening methods. Therefore, the observed biological effects are more likely attributable to the overall phytochemical composition of the extract rather than to root-specific constituents.

The high concentration of flavonoids (+++) suggests that they may be the primary contributors to the observed trend. Flavonoids are well-documented phytoestrogens that can bind to estrogen receptors and modulate reproductive hormone signaling, potentially shifting the maternal hormonal milieu [10,11]. This shift can influence the selective survival of male embryos or the receptivity of the reproductive tract to Y-carrying sperm [12,19]. Furthermore, the moderate presence of cardiac glycosides (++) is noteworthy, though their specific contribution to the observed reproductive effects remains unclear and warrants further investigation. The 10% increase in male offspring (55.8% vs 45.8%), while not statistically significant, suggests a possible biological trend that is consistent with ethnobotanical claims (93.9% success rate).

Potential mechanisms include hormonal Modulation: Phytoecdysteroids and flavonoids may alter the maternal estrogen/androgen balance, potentially affecting sperm-ovum interactions or early embryonic survival [10,12,20]. Oxidative Stress Reduction: Flavonoids may protect male embryos, which are often more vulnerable to oxidative damage during early

development [19]. Metabolic Influence: Phytochemicals may indirectly affect sex determination by modulating maternal glucose or lipid metabolism, consistent with the Trivers-Willard hypothesis [14,15,21].

Limitations

The main limitation of this pilot study is the small sample size (n=8), which limits the statistical power to detect modest effects. Future research should include larger cohorts, quantitative phytochemical analysis (HPLC), and hormonal profiling (Estrogen, Progesterone, Testosterone) to provide a mechanistic interpretation of the observed trend.

Conclusion

Silene apetala is a plant traditionally used by some women in Libya with a reputation for influencing offspring sex. This study documented its traditional use and identified bioactive flavonoids and glycosides. While the experimental increase in male offspring was not statistically significant, the 10-percentage-point shift represents a biological trend that warrants further investigation.

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Conflict of Interest

The authors declare no conflict of interest.

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References

1. World Health Organization. WHO global report on traditional and complementary medicine 2019. Geneva: World Health Organization; 2019. Available from: <https://www.who.int/publications/i/item/978924151536>
2. Kasilo OMJ, Wambebe C, Nikiema JB, Nabyonga-Orem J. Towards universal health coverage: advancing the development and use of traditional medicines in Africa. *BMJ Glob Health*. 2019;4(Suppl 9): e001517. doi: 10.1136/bmjgh-2019-001517
3. Hassan SA, El Werfaly AD, Asker AM. Floristic Composition, Chorotypes and Life form of the Family Caryophyllaceae, Libya. 2025 Jul 6; Available from: <https://qlaj.elmergib.edu.ly/index.php/qlaj/article/view/67>
4. Mamadalieva NZ, Koval A, Dusmuratov MM, Hussain H, Katanaev VL. Chemical and Biological Characterization of Metabolites from *Silene viridiflora* Using Mass Spectrometric and Cell-Based Assays. *Biomolecules*. 2024;14:1285. doi: 10.3390/biom14101285
5. Dürüst N, Dürüst Y, İkinci N, Banko S, Hoşgün EZ, Bozan B. HPLC determination of polyphenols of the flowers of *Digitalis lamarckii*, *Xeranthemum annuum*, *Epilobium hirsutum* and *Silene compacta* from Bolu (Turkey). *J Med Plants Res*. 2023;17(4):164-179. doi:10.5897/JMPR2022.7282.
6. Mamadalieva NZ, Lafont R, Wink M. Diversity of Secondary Metabolites in the Genus *Silene* L. (Caryophyllaceae) – Structures, Distribution, and Biological Properties. *Diversity*. 2014;6(3):415-499. doi: 10.3390/d6030415
7. Mamadalieva NZ. Phytoecdysteroids from *Silene* plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants [Internet]*. 2012;11:474-497 Available from: <https://www.redalyc.org/pdf/856/85624607001.pdf>
8. Ramazonov NS, Yusupova UY, Egamova FR, et al. Anti Stress Activity of Phytoecdysteroids Isolated from Aerial Part of *Silene claviformis*. *Chem Sci Int J*. 2020;29(7):1-8. doi: 10.9734/csji/2020/v29i730190
9. Sidana J, Devi RR, Kumar P, Singh B, Sharma OP. Phytoecdysteroid Profiling of *Silene vulgaris* by UPLC-ESI-MS. *Current Science*. 2017;113:1986. doi: 10.18520/CS/V113/I10/1986-1992.

10. Retana-Márquez S, Hernández H, Flores JA, Muñoz-Gutiérrez M, Duarte G, Vielma J, Fitz-Rodríguez G, Fernández IG, Keller M, Delgadillo JA. Effects of phytoestrogens on mammalian reproductive physiology. *Tropical and Subtropical Agroecosystems* [Internet]. 2011;15.
11. Jefferson WN, Patisaul HB, Williams CJ. Reproductive consequences of developmental phytoestrogen exposure. *Reproduction*. 2012;143(3):247-260. doi: 10.1530/REP-11-0369
12. Sarma I, Gowala U, Misra K, Saikia A, Saikia AJ, Namchoom K, Sarma HN. Beneficial Endocrine Disruption as Steroid Receptor Functional Modulator by Phytocompounds from Medicinally Important Plants: Impact on Female Reproduction Regulation. 2021;1-9. doi: 10.1007/S12595-021-00404-3.
13. Lambert MR, Giller GSJ, Barber LB, Fitzgerald KC, Skelly DK. Hormonally active phytochemicals and vertebrate evolution. *Evol Appl*. 2017;10(7):632-644. <https://doi.org/10.1111/eva.12469>
14. Rosenfeld CS, Roberts RM. Maternal diet and other factors affecting offspring sex ratio: A review. *Biol Reprod*. 2004;71(4):1063-1070. <https://doi.org/10.1095/biolreprod.104.030890>
15. Cameron EZ. Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. *Proc R Soc B*. 2004;271(1549):1723-1728. <https://doi.org/10.1098/rspb.2004.2773>
16. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall; 1998.
17. Trease GE, Evans WC. *Pharmacognosy*. 15th ed. London: WB Saunders; 2002.
18. Zibareva L, Volodin V, Saatov Z, Savchenko T, Whiting P, Lafont R, et al. Distribution of phytoecdysteroids in the Caryophyllaceae. *Phytochemistry*. 2003;64(2):499-517. doi:10.1016/S0031-9422(03)00364-X
19. Guérin P, El Mouatassim S, Ménézo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update*. 2001;7(2):175-189. doi:10.1093/humupd/7.2.175
20. Lafont R, Dinan L. Practical uses for ecdysteroids in mammals including humans: an update. *J Insect Sci*. 2003;3:7. doi:10.1093/jis/3.1.7
21. Trivers RL, Willard DE. Natural selection of parental ability to vary the sex ratio of offspring. *Science*. 1973;179(4068):90-92. doi:10.1126/science.179.4068.90