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Cytogenetic characterization of *Guaiacum officinale*, chromosome number and conservation management

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Abstract. *Pikulthong V, Javadi B, Maneechai S, Homthong M, Sarakit P, Thongprapha C, Umpunjun P, Sraphet S, Tanomtong A. 2025. Cytogenetic characterization of* Guaiacum officinale*, chromosome number and conservation management. Biodiversitas 26: 5468-5475.* *Guaiacum officinale*, commonly known as lignum vitae (*Kaew Chao Chom*), is a tree species in the Zygophyllaceae family valued for its ecological and economic importance but threatened by overexploitation. Cytological studies of this endangered species remain scarce. This study presents the first cytogenetic analysis of *G. officinale*, focusing on both mitotic and meiotic processes. Root tip samples were collected between 05:00 and 12:00 to examine mitosis, while young flower buds were collected between 06:00 and 13:00 to analyze meiosis. Peak mitotic activity occurred at 10:00, although the very small chromosome size and limited spreading during slide preparation posed challenges for accurate counting. In contrast, meiotic analysis at 08:00 provided clear evidence of 12 bivalents at metaphase I, establishing a diploid chromosome number of 2n = 2x = 24. Chromosome pairing was generally regular, with occasional anomalies observed, but overall meiotic stability indicated high fertility and strong reproductive potential. These findings clarify the chromosomal behavior of *G. officinale* and demonstrate its diploid, cytologically stable nature. The results provide a critical baseline for understanding taxonomy and evolutionary relationships within Zygophyllaceae and offer essential guidance for conservation and sustainable management of this endangered species. These findings represent the first cytogenetic report of *G. officinale* in Thailand and provide baseline data for future taxonomic, genetic, and conservation studies of this threatened species.

Keywords: Chromosomes, *Guaiacum officinale*, *Kaew Chao Chom*, meiosis, Zygophyllaceae

INTRODUCTION

Guaiacum officinale L. (lignum vitae), known in Thai as *Kaew Chao Chom*, is a member of the Zygophyllaceae family. This evergreen tree is native to South America and the West Indies (Kotresha and Savitha 2004). The Zygophyllaceae comprise 27 genera and 285 species of herbs and shrubs (Gupta et al. 2016). Members of the family possess simple or compound leaves, arranged alternately or oppositely, often with stipules. Their flowers are typically blue to purple with 4-5 sepals and petals, 4-5 stamens, and 4-5 carpels, producing fruits that are either capsules or drupes (Sheahan and Chase 2000; Pendyala et al. 2017; Zeb et al. 2017). In Thailand, two genera from this family are present: *Tribulus* (with *T. cistoides* and *T. terrestris*) and the introduced *G. officinale* (Chayamarit 1985). *G. officinale* has long been valued for its exceptionally dense wood, which is resistant to seawater and suitable for marine construction and fine carvings. Its floral design has influenced Benjarong porcelain art in Thailand (Maneechai and Pikulthong 2017). Medicinally, the plant is rich in saponins such as guaiacin, traditionally used for treating phlegm, arthritis (Pendyala et al. 2017; Promsorn 2019), inflammation, and cholesterol disorders (Amini-Chermahini et al. 2014; Maneechai and Pikulthong 2017; Wiart 2021; Foster 2023; Oakeley 2023; Prasalini and Kumar 2023). More recent studies report anticancer, anti-infective, and antioxidant properties (Saba et al. 2012), including activity against HIV-1 (Ahmad et al. 2000; Ahmad et al. 2004; Lowe et al. 2014; Sarkar et al. 2014). It was also utilized as a dye in traditional ceramic production (Evans and Evans 2009; Botticelli et al. 2021).

Due to centuries of overharvesting for wood, medicine, and dye production, natural populations of *G. officinale* have sharply declined, leading to its classification as Endangered under IUCN Red List criteria A2d (Barstow 2019). International trade is regulated under CITES Appendix II, and the species is strictly protected in Thailand (Groves and Rutherford 2023). Despite its ecological and economic significance, scientific knowledge of its genetics and cytology remains scarce, hindering conservation planning. Cytological studies, particularly those addressing mitosis and meiosis, are vital for understanding chromosome behavior, genetic stability, and reproductive potential in

endangered plants. They provide insights into species diversity and adaptability, while linking with morphology, anatomy, palynology, and molecular markers to refine taxonomy and resolve phylogenetic ambiguities (Rahimi et al. 2011). Chromosome numbers and structures are especially informative as taxonomic markers, distinguishing closely related taxa and revealing evolutionary processes. Within Zygophyllaceae, chromosome investigations are limited to a handful of genera (*Tribulus*, *Zygophyllum*, *Fagonia*, and *Peganum*), showing diverse base numbers (x = 6, 10, 11, or 12) and ploidy levels. However, no cytogenetic studies have previously been reported for *G. officinale*, despite its endangered status and economic importance. Its restricted distribution and declining populations highlight the urgent need for science-based conservation strategies. Cytogenetic studies, particularly those on chromosome numbers and meiotic stability, provide crucial insights into the genetic integrity and reproductive success of threatened plants. Without such information, conservation planning risks being incomplete, leaving species vulnerable to further decline. Establishing a cytogenetic baseline for *G. officinal*e, therefore, represents a critical step toward ensuring its long-term survival and sustainable utilization.

The present study, provides the first cytogenetic characterization of *G. officinale* in Thailand. By examining mitotic and meiotic divisions, we aimed to determine its chromosome number, assess meiotic stability, and highlight methodological challenges in chromosome visualization. These findings not only fill a major knowledge gap in Zygophyllaceae cytogenetics but also establish a cytological baseline that can enlighten taxonomy, phylogeny, and conservation management of this endangered species.

MATERIALS AND METHODS

Sample collection and plant identification

Specimens of *Guaiacum officinale* were collected from Suan Sunandha Rajabhat University and subsequently deposited at the Forest Botany Herbarium, which is part of the Forest and Plant Conservation Research Office under the Department of National Park, Wildlife, and Plant Conservation. This association highlights the importance of preserving and studying plant biodiversity, as these specimens play a significant role in various research initiatives and conservation efforts. By depositing the specimens in a recognized herbarium, they are ensured to be carefully curated, cataloged, and made accessible for future scientific studies. The morphology of *G. officinale*—including its flowers, leaves, ripe fruit, and seeds—is depicted in Figure 1.

Mitotic and meiotic chromosomal analysis

Chromosomal analysis of mitotic and meiotic cells was performed using the aceto-orcein squash technique, with some modifications based on the protocols established by Nopporncharoenkul et al. (2017).

Mitotic chromosome analysis

Seeds of *G. officinale* were germinated in a petri dish containing sterile cotton and then transplanted into sterile soil for 1-2 weeks (Figures 2.B-2.C). After germination, actively growing root tips, approximately 2 cm in length, were collected hourly from 05:00 to noon for pretreatment. The root tips were treated with a saturated solution of α-bromonaphthalene at 10°C for 4-12 hours to arrest cell division and facilitate chromosomal counting.

Immediately following the treatment, the root tips were fixed in Carnoy's fixative solution (3:1 v/v mixture of ethanol and glacial acetic acid) at 10°C for 24 hours to preserve cellular structures. The acidic properties of this fixative prevent nucleoprotein precipitation and the dissolution of cytoplasmic proteins, ensuring the integrity of the cell components. After fixation, the root tips were rinsed twice with distilled water to remove any residual fixative.

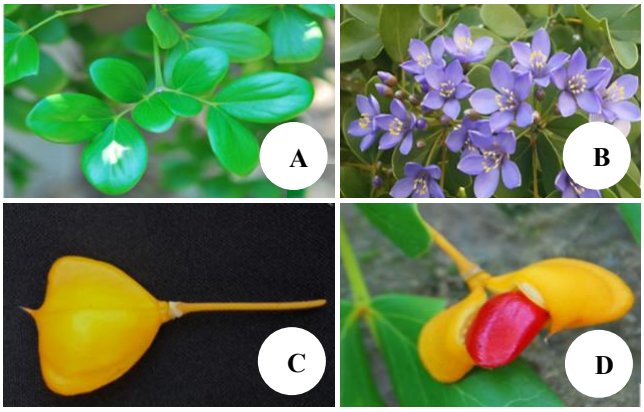

Figure 1. The morphology of *Guaiacum officinale* in Thailand: A. Leaves, opposite, paripinnate. Leaflets 2-5 pairs, obovate, rounded at apex, obtuse or cuneate at base, coriaceous glossy. B. Purple flower clustered, sepals 5, pubescent. C. Ripe fruit are yellow, heart-shaped, compressed, with two wing-like angles, 2-celled. D. Mature seeds covered by a fleshy, red aril emerge from ripe fruit

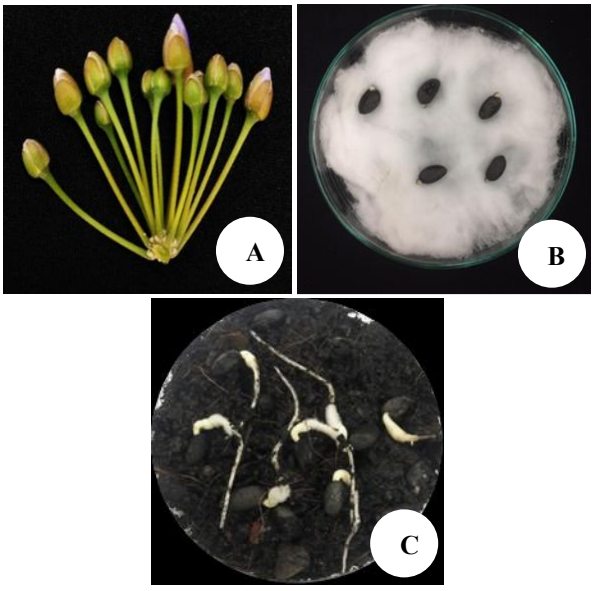

Figure 2. The specimens of *Guaiacum officinale*: A. Young flower buds and anthers, B. Seeds cultivated in a petri dish, and C. Germinating root tips in a petri dish

The root tips were then hydrolyzed in 1 N HCl in a water bath at 60°C for 9 minutes. Following hydrolysis, the root tips were directly transferred to a 2% (w/v) aceto-orcein solution for staining. The stained root tips were squashed on a microscope slide to spread the cells into a single layer, using an adapted Feulgen squash technique (Sharma and Sharma 2014; Nopporncharoenkul et al. 2017).

The metaphase stages, where chromosomes align at the center of the cell, were observed under a light microscope at a magnification of 100× in at least 20 cells to count the number of chromosomes. Chromosomal parameters were measured in 5 cells, and images of the cell divisions were captured using an Olympus DP50 microscope camera.

Meiotic chromosome analysis

Young inflorescences of *Guaiacum officinale*, containing predominantly closed flower buds, were collected between 06:00 and 13:00 (Figure 2.A). Samples were taken hourly during this period and immediately fixed in Carnoy's fixative solution (a 3:1 v/v mixture of ethanol and glacial acetic acid) at 4°C for 48 hours. This fixation process halted all cellular activities and reactions, preserving the cellular structures. The fixed samples were rinsed with 95% alcohol and then transferred to 70% alcohol for storage at 4°C until further use.

Before proceeding with the analysis, the samples were rehydrated in distilled water for two 10-minute intervals. Under a stereomicroscope, each anther from the flower buds, containing Pollen Mother Cells (PMCs) (Figure 2.A), was carefully isolated from unwanted parts of the closed flower. The isolated anthers were stained using a conventional technique with 2% (w/v) aceto-orcein.

The prepared microscopic slide was briefly passed through the flame of a burner three to five times. The warm anther suspension was gently spread with dissecting needles, and any remaining tissue debris was meticulously removed. The resulting fine cell suspension was covered with a coverslip and compressed to form a single cell layer on the microscope slide.

Chromosomes were observed during metaphase, aligning in the center of the cell, under a light microscope at 100x magnification in at least 20 cells to count the number of chromosomes. Chromosomal parameters were measured in 5 cells, and the images of cell divisions were captured using an Olympus DP50 microscope camera.

RESULTS AND DISCUSSION

Mitotic chromosome

Root tips of *Guaiacum officinale* were collected hourly from 05:00 to 12:00 to examine mitotic cell division. The highest mitotic activity was observed at 10:00, indicating this as the optimal period for chromosome observation. Due to the small size and compact nature of the chromosomes, high-quality metaphase spreads were relatively uncommon. However, in a subset of well-spread metaphase cells, a diploid chromosome number of 2n = 2x = 24 was observed, providing limited but concordant evidence with the meiotic findings. No mitotic abnormalities, such as lagging chromosomes, chromatin bridges, or micronuclei, were detected in the cells examined (Figure 3).

Meiotic chromosome

Anthers (microsporocytes) of *Guaiacum officinale* were collected hourly, from 06:00 to 13:00, to study cell division during meiosis. The results indicated that 8:00 AM was the peak time for meiosis, making it the most suitable period for observing cell division. Figure 4 presents the meiotic analysis of *G. officinale* using the conventional aceto-orcein staining method. During diakinesis, which occurs between late prophase I and early metaphase I (Figure 5.A), 12 pairs of homologous chromosomes, known as bivalents (12II), were observed forming a regular meiotic structure (Figures 5.B-5.D). The chromosomal evidence suggests that *G. officinale* is diploid, containing a basic chromosome number of 2n = 2x = 24. Successive stages of meiosis were recorded, including telophase I (E), prophase II (F), metaphase II (G), anaphase II (H), telophase II (I), tetrad formation (J), and mature pollen grains (K). Although meiosis was generally regular, occasional anomalies such as lagging chromosomes and uneven segregation were observed, which may influence pollen development and fertility.

Discussion

Technical challenges in cytology of Guaiacum officinale

The study of mitotic cell division in young root tips of *G. officinale* using the conventional aceto-orcein staining method presented challenges. The technique resulted in poor contrast between the chromosomes and cytoplasm, making it difficult to determine the number and size of chromosomes. This difficulty arose because *G. officinale* chromosomes are relatively small. Additionally, the thicker cell walls of *G. officinale* compared to other plants likely contributed to the poor dispersion and visualization of chromosomes during metaphase. Therefore, adjustments to the hydrolysis method and duration were necessary to break down the cell walls better and improve chromosome visibility. The mitotic analysis faced difficulties due to poor chromosomal spreading and low contrast, common in woody species with thick cell walls and small chromosomes. Similar technical constraints have been reported in other cytogenetic studies (Amini-Chermahini et al. 2014). Despite these challenges, the limited mitotic counts that were obtained aligned with the meiotic results, confirming the diploid chromosome number. Future studies could employ advanced methods such as flow cytometry, Fluorescence In Situ Hybridization (FISH), or improved pretreatment protocols to generate higher-quality mitotic spreads and enable karyotype-level analyses.

Previous cytological studies on medicinal and alpine plants have shown that meiotic behavior is a critical determinant of reproductive success and pollen fertility. For example, Jeelani et al. (2015) reported considerable variation in morphology, ecology, and chromosomes in *A. heterophyllum*, where meiotic irregularities such as univalents and laggards were associated with reduced pollen fertility. Similarly, Jeelani et al. (2014) conducted detailed investigations on polypetalous plants from the

Kashmir Himalaya and highlighted the occurrence of meiotic abnormalities, including chromosomal stickiness and bridges, which directly affected pollen viability. Kumar et al. (2013), in their cytological analysis of five Papaveroideae species, also emphasized that meiotic irregularities often lead to reduced gametic fertility and reproductive constraints in wild plant species. In contrast, our study revealed regular meiotic pairing and high pollen fertility in *G. officinale*, indicating cytogenetic stability and strong reproductive potential. This finding underscores the importance of stable chromosome behavior for the conservation and management of endangered plant species. This suggests that meiotic stability plays a key role in maintaining the reproductive competence and conservation potential of this endangered medicinal plant.

Chromosome number in the context of Zygophyllaceae

This study provides the first cytogenetic characterization of *G. officinale* in Thailand, establishing a diploid chromosome number of 2n = 2x = 24 based on clear meiotic observations of 12 bivalents at metaphase I (Table 1). Limited but concordant mitotic counts were obtained from well-spread root tip cells, though small and compact chromosomes posed technical challenges for routine mitotic analysis. These findings close an important knowledge gap for this endangered species, whose cytology had not previously been reported from the region.

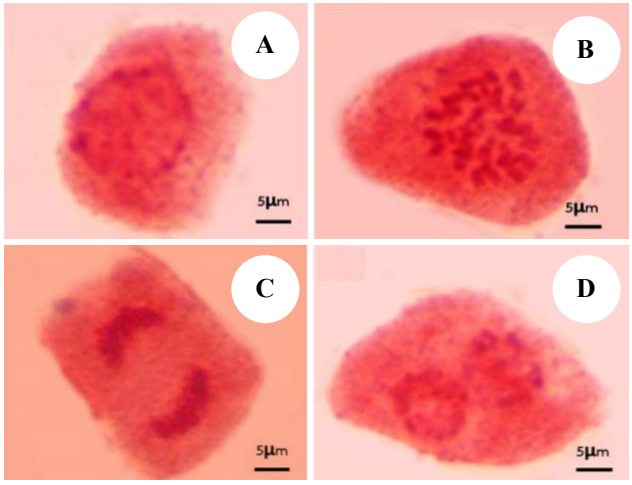

Figure 3. Mitotic cell division from the root tips of *Guaiacum officinale*: A. Prophase, B. Metaphase, C. Anaphase, and D. Telophase. Scale bar: 5 µm

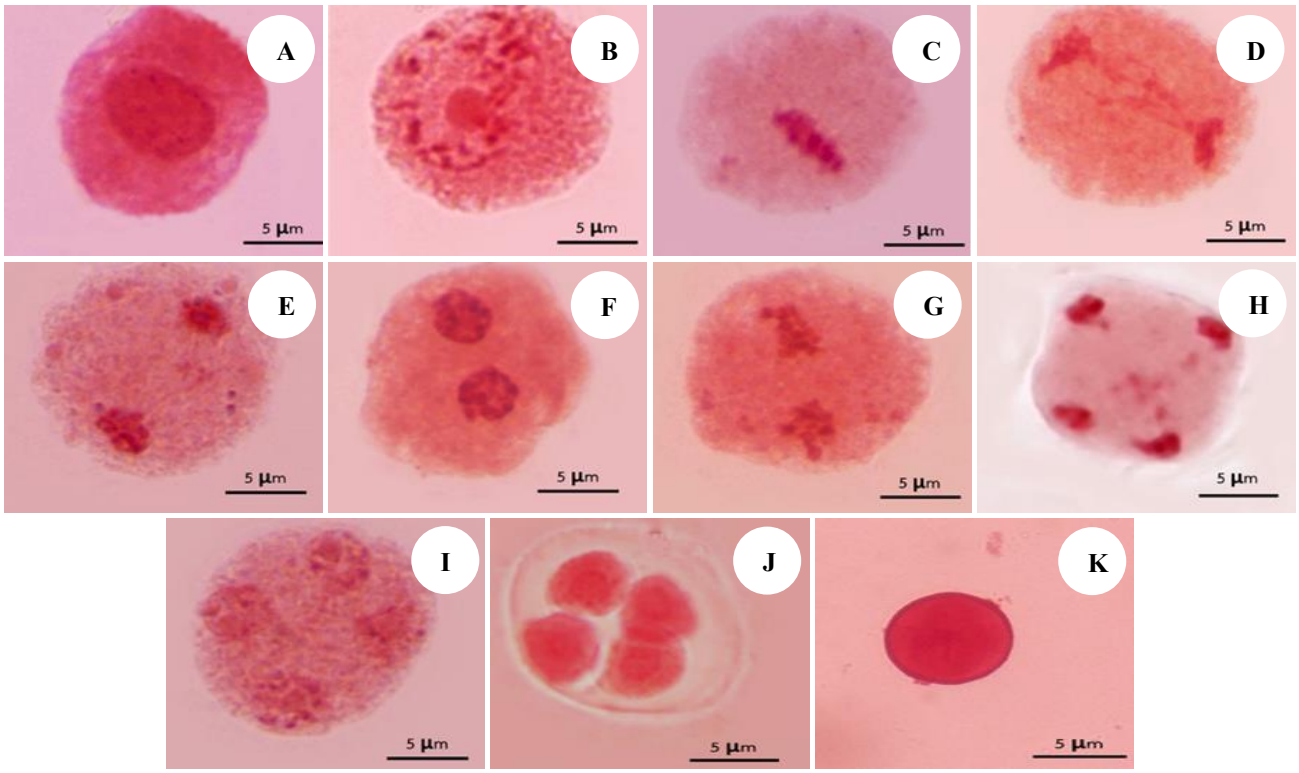

Figure 4. Meiosis cell division of *Guaiacum officinale* (anthers): A. Interphase, B. Prophase I, C. Metaphase I, D. Anaphase I, E. Telophase I, F. Prophase II, G. Metaphase II, H. Anaphase II, I. Telophase II, J. Tetrad cell, and K. Pollen. Scale bar: 5 µm

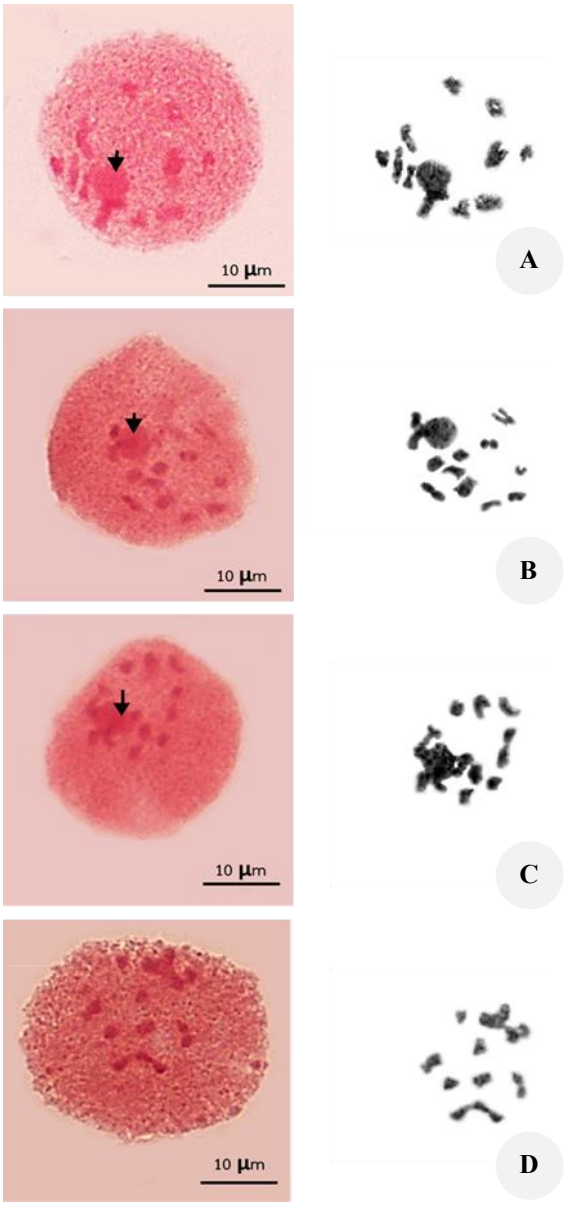

Figure 5. Chromosome shape of *Guaiacum officinale*, derived from meiosis cell division of microsporocytes; A-C: Diakinesis 2n = 24, D. Metaphase I showed bivalents = 12, and the arrow in Figures 5.A-5.C refers to the nucleolus. Scale bar: 10 µm

In comparison with other studies on the Zygophyllaceae family, such as those by Amini-Chermahini et al. (2014), which examined the chromosome numbers of *Zygophyllum eichwaldii* C.A.Mey. and *Zygophyllum eurypterum* Boiss. & Buhse, the results indicated that the basic chromosome number for these species was x = 11 (Liu et al. 2001). Specifically, *Z. eichwaldii* had a diploid number of 2n = 2x = 22, while *Z. eurypterum* had a tetraploid number of 2n = 4x = 44. Gupta et al. (2016) reported the first chromosome studies of *Fagonia cretica*, *Tribulus alatus*, and *Zygophyllum simplex*, presenting a basic chromosome number of x = 6 for these species. However, recent research from India has shown that the basic chromosome number for these species might be x = 12, with *F. cretica* having a chromosome number of 2n = 22, *T. alatus* having 2n = 24, and *Z. simplex* having 2n = 16 (Gupta et al. 2016). These dissimilarities highlight the difficulty and diversity of chromosome numbers within the Zygophyllaceae family. Chromosome numbers within the Zygophyllaceae family show considerable variation, ranging from diploid to polyploid levels (Amini-Chermahini et al. 2014; Gupta et al. 2016). For example, *Tribulus terrestris* displays 2n counts from 24 to 48 across cytotypes (Rawat et al. 2006), while Zygophyllum species exhibit 2n = 16-44 (Morsy and El Sherbeny 2015). Despite this variation, a base number of x = 12 is frequently reported in many polypetalous genera, as summarized by Rani et al. (2014). The confirmation that *G. officinale* conforms to this base number supports its taxonomic placement within the family and suggests cytogenetic stability without evidence of recent polyploidization.

Meiotic stability and reproductive potential

Additionally, the study of meiotic chromosomes and homologous chromosome pairing in *G. officinale*, investigated using the conventional aceto-orcein staining technique, successfully identified all stages of cell division. This analysis discovered 12 bivalents homologous chromosomes, confirming a chromosome number of 2n = 2x = 24. The basic chromosome number of x = 12 shows that *G. officinale* is a diploid and fertile species. Similar studies on alpine and medicinal plants have demonstrated that meiotic regularity-particularly bivalent formation and absence of chromosomal irregularities—is closely linked to pollen fertility and reproductive success (Kumar et al. 2013; Jeelani et al. 2014, 2015). In *G. officinale*, the predominance of regular meiotic divisions and the presence of well-formed pollen grains indicate high gametic fertility, an essential feature for maintaining viable natural populations. From a conservation perspective, the high pollen fertility inferred from cytological stability is encouraging. Endangered species often show meiotic irregularities linked to genetic erosion or habitat stress (Jeelani et al. 2015), yet *G. officinale* appears to retain strong reproductive potential.

Implications for taxonomy and conservation

Cytological analysis is cornerstone of scientific research, providing critical insights into chromosomal behavior, genetic variation, and evolutionary processes. This work highlights the first cytological report on *G. officinale*, an endangered species of economic and ecological value belonging to the family Zygophyllaceae. Despite its importance, comprehensive cytological studies on this species have been limited, making this research an important addition to scientific understanding.

Table 1. Diploid chromosome number (2n), Fundamental Number (FN), and ploidy level (x) of family Zygophyllaceae in previous reports

Species	2n	FN	Ploidy level (x)	Reference
Fagonia cretica L.	18	-	-	(Baquar 1967)
	20	-	-	(Baquar 1967; Bhansali 1974)
	22	-	2x	(Baquar 1967; Bhansali 1974; Amini-Chermahini et al. 2014; Gupta et al. 2016; Marhold and Kučera 2016)
Fagonia schweinfurthii (Hadidi) Hadidi	22	-	-	(Marhold and Kučera 2016)
Guaiacum officinale L.	24	12	2x	First report
Peganum harmala L.	12	6	2x	(Gupta et al. 2016; Marhold and Kučera 2016)
	22	6	2x	(Xing-Hua et al. 1984)
	24	-	2x	(Löve 1973; Koul and Wakhlu 1976; Magulaev 1979; Singh 1984; Aslam et al. 2023)
	48	-	4x	(Aslam et al. 2023)
Tribulus alatus Delile	12	-	4x	(Agarwal and Roy 1976)
	24	-	4x	(Agarwal and Roy 1976; Hilu 1981; Gupta et al. 2016)
Tribulus micrococcus Domin	48	6, 12	-	(Morrison and Scott 1996)
Tribulus occidentalis R.Br.	48	6, 12	-	(Morrison and Scott 1996)
Tribulus rajasthanensis Bhandari & V.S.Sharma	24	12	-	(Bhandari and Sharma 1977; Rawat et al. 2006)
Tribulus terrestris L.	24	12	4x	(Sugiura 1940; Heiser Jr and Whitaker 1948; Bhansali 1974; Hilu 1981; Gupta et al. 2016)
	36	-	6x	(Hilu 1981; Antil et al. 2023)
	48	-	8x	(Singh 1984; Gupta et al. 2016)
	24, 36, 48	-	4x, 6x, 8x	Morrison (Morrison and Scott 1996; Al-Turki et al. 2000)
Zygophyllum coccineum L.	16	-	-	(Al-Turki et al. 2000)
	18	-	-	(Eid 1970)
	32	-	-	(Morsy and El Sherbeny 2015)
Zygophyllum decumbens Delile	24	-	-	(Eid 1970)
Zygophyllum dumosum Boiss.	24	-	-	(Morsy and El Sherbeny 2015)
Zygophyllum eichwaldii C.A.Mey.	22	11	2x	(Amini-Chermahini et al. 2014)
Zygophyllum eurypterum Boiss. & Buhse	44	11	4x	(Amini-Chermahini et al. 2014; Samaras et al. 2021)
Zygophyllum fabago L.	22	-	2x	(Amini-Chermahini et al. 2014, 2017)
Zygophyllum simplex L.	16	-	2x	(Morsy and El Sherbeny 2015; Gupta et al. 2016)

Note: 2n: Diploid chromosome number, FN: Fundamental Number, x: Basic chromosome number, -: Non valid

Chromosome numbers and meiotic behavior are widely recognized as reliable taxonomic markers (Guerra 2008). The regular bivalent formation and absence of structural abnormalities in *G. officinale* confirm genetic stability, supporting its classification and providing baseline data for molecular phylogenetic studies. Establishing a stable diploid count for *G. officinale* provides a cytogenetic baseline for future studies on genome organization, population genetics, and phylogeny. Combined with molecular tools, these data can guide ex situ conservation, genetic improvement, and sustainable utilization strategies. Advances in cytological techniques such as high-resolution microscopy, molecular cytogenetics, and bioinformatics will further strengthen future studies.

The confirmation of diploid chromosome number (2n = 2x = 24) and the predominance of regular meiotic behavior in *G. officinale* highlight the species' genetic stability, which is a critical foundation for long-term conservation. High meiotic stability ensures reliable gamete formation and pollen fertility, reducing the risk of reproductive failure that often afflicts endangered species. This stability directly supports the success of both natural regeneration and ex situ conservation programs, such as botanical garden collections or seed banks. By establishing a cytogenetic baseline, this study provides essential reference data for monitoring potential genetic erosion in fragmented populations and for guiding reintroduction or restoration initiatives. Furthermore, the evidence of strong reproductive biology implies that conservation strategies can prioritize habitat protection and sustainable propagation without immediate concern for cytogenetic irregularities. Importantly, integrating cytogenetic data with molecular tools will allow conservationists to trace genetic diversity across populations, ensuring that ex situ efforts capture the full range of genetic variation necessary for resilience to environmental change. Thus, the cytological characterization of *G. officinale* is not only a taxonomic contribution but also a pivotal step toward securing the evolutionary future of this endangered and ecologically valuable species.

This study confirms that *G. officinale* possesses a diploid chromosome number of 2n = 2x = 24, inferred from regular meiotic behavior with 12 bivalents and supported by limited mitotic evidence. The results align with previous reports on chromosome diversity in the Zygophyllaceae family and highlight the species' genetic stability, high fertility, and conservation potential. These findings provide essential cytogenetic insights for taxonomy, reproductive biology, and long-term management of this endangered plant.

In conclusion, this study provides the first comprehensive cytogenetic characterization of *G. officinale*, establishing its diploid chromosome number (2n = 2x = 24) and confirming stable meiotic behavior with regular bivalent formation. These findings contribute valuable taxonomic evidence that supports the placement of *G. officinale* within the Zygophyllaceae and provide baseline data for resolving phylogenetic relationships in the family. From a conservation perspective, the confirmation of genetic stability and high reproductive potential offers reassurance that viable populations persist, while also underscoring the urgency of protecting this endangered species from further decline. The cytogenetic baseline established here will serve as a foundation for ex situ conservation programs, restoration efforts, and the integration of molecular approaches to track genetic diversity across populations. However, this study was limited by its small sample size and reliance on conventional aceto-orcein staining, which may restrict detailed karyotype resolution. The analysis was confined to a single population, preventing assessment of intraspecific chromosomal variation. Future research should therefore incorporate advanced molecular cytogenetic methods such as Fluorescence In Situ Hybridization (FISH) or flow cytometry, along with broader population sampling, to validate and extend these baseline findings.

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