

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 P-ISSN: 2349-8234 https://www.phytojournal.com JPP 2024; 13(2): 229-232 Received: 02-02-2024 Accepted: 10-03-2024

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Integrating morphology, anatomy, and preliminary phytochemistry for distinguishing *Cuscuta reflexa* and *Ziziphus jujuba*

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DOI: https://doi.org/10.22271/phyto.2024.v13.i2c.14883

Abstract

Accurately distinguishing parasitic plants from their hosts remains a challenge due to potential morphological convergence. This study tackles this by employing a multifaceted approach integrating morpho-anatomical features and preliminary phytochemical analysis for the precise differentiation of *Cuscuta reflexa*, a parasitic climber, and its host *Ziziphus jujuba*. A comparative analysis of vegetative and reproductive morphology revealed stark differences, including the complete absence of stems in *C. reflexa* contrasting with the well-developed woody stems of *Z. jujuba*. Leaf shape, venation patterns, and floral structures further supported their distinction. Light microscopy investigations of stem and leaf tissues delineated variations in tissue organization, vascular bundle arrangement, and the presence/absence of trichomes. Furthermore, a comprehensive preliminary phytochemical screening yielded contrasting profiles, highlighting the presence of specific secondary metabolites unique to each species. This combined approach effectively differentiated the parasite from its host, providing valuable insights for accurate plant identification in various fields like taxonomy, ethnobotany, and ecological studies of parasitic interactions. The identified distinguishing features contribute to a more comprehensive understanding of host-parasite relationships.

Keywords: Cuscuta reflexa, Ziziphus jujuba, parasitic plants, host identification, Morpho-anatomy, phytochemical screening, species differentiation

Introduction

The intricate relationships between parasitic plants and their host species have captivated botanists, ecologists, and agriculturists for centuries (Gibson & Watkinson, 1991)^[8]. Unlike typical autotrophic plants that synthesize their own food, parasitic plants have evolved unique strategies to exploit the resources of other plants, known as hosts (Nickrent & Musselman, 2004)^[14]. This complex interaction involves a dynamic exchange of nutrients, water, and signals, ultimately shaping the ecology and physiology of both the parasite and the host (Press & Graves, 1995)^[17].

Parasitic plants constitute a diverse group encompassing various families and genera, displaying a range of adaptations for their parasitic lifestyle (Hawksworth & Sutton, 2000)^[9]. Unlike autotrophs, parasitic plants lack chlorophyll and rely entirely or partially on hosts for their nutritional requirements (Cameron *et al.*, 2009)^[3]. This dependence is facilitated by specialized structures called haustoria, which establish connections with the host, enabling the transfer of resources (Parker & Riches, 1993)^[16].

Host plants, on the other hand, play a crucial role in this symbiotic interaction. They provide the necessary resources for the parasitic plant's survival, often undergoing physiological and anatomical changes in response to parasitism (Yoder *et al.*, 2010)^[21]. The relationship between parasitic and host plants can range from mutualistic to detrimental, depending on the specific species involved and environmental factors (Atsatt & Sanders, 1993)^[2].

One fascinating example of parasitic plants is the genus Cuscuta, commonly known as dodder (Norton, 1977)^[15]. Cuscuta species are characterized by their slender, thread-like stems and the absence of leaves and roots. Lacking chlorophyll, Cuscuta relies entirely on its host for sustenance (Dörfler *et al.*, 2016)^[5]. The plant establishes a connection with the host through haustoria, specialized structures that penetrate the host's tissues (Hawksworth & Sutton, 2000)^[9]. In this study, we focus on *Ziziphus jujuba*, a common host species for Cuscuta. As with any host plant, *Ziziphus jujuba* plays a critical role in the dynamics of the parasitic relationship. Understanding the morphological, anatomical, and phytochemical aspects of *Ziziphus jujuba*

and its interactions with Cuscuta contributes to our broader comprehension of plant-plant interactions (Yoder *et al.*, 2010) [21].

Materials and Methods

Plant material collection

Mature specimens of *Cuscuta reflexa* (parasite) and its host *Ziziphus jujuba* were collected from forest field area of Jalna districts, Maharashtra, India and Plant identification was confirmed by Dr. Umesh P. Mogle, professor and Head Dept. of Botany, JES College, Jalna (MS). Herbarium specimen of both plants *Cuscuta reflexa* and *Ziziphus jujuba* were deposited in the dept. of Botany. The collection process ensured inclusion of both vegetative and reproductive parts for comprehensive analysis.

Morphological analysis

Detailed morphological observations of both *C. reflexa* and *Z. jujuba* were conducted. This included examining vegetative features like stem presence/absence, stem diameter and color, leaf shape, size, venation patterns, and presence/absence of trichomes. Reproductive structures like flower morphology, arrangement, and inflorescence type were also documented using a magnifying glass and digital camera with appropriate scale bars. Photographs were captured to create a comprehensive morphological record of both species.

Anatomical analysis

Fresh stem and leaf tissues from both *C. reflexa* and *Z. jujuba* were collected for anatomical studies. Tissue samples were sectioned using a microtome. The sections were then stained and mounted on glass slides for microscopic observation. Anatomical features like tissue organization, presence/absence of specific cell types (e.g., sclerenchyma, collenchyma), vascular bundle arrangement, and trichome morphology were examined using a light microscope at various magnifications. Microscopic images were captured for documentation and comparison.

Preliminary phytochemical screening

C. reflexa and *Z. jujuba* were separated from the eachothers, washed, shade-dried (6-8 days), and ground into powder. Eighty grams were macerated in 400 mL ethanol (48 h, occasional stirring). The extract was filtered (muslin cloth, Whatman No. 1) and evaporated to dryness (40°C). Extract yield and color were recorded. The extract was stored at 4°C. Phytochemical analysis for secondary metabolites was done by following phytochemical tests Shaikh & Patil (2020)^[20].

Data analysis

The data obtained from morphological analysis, anatomical observations, and preliminary phytochemical screening were compiled and analyzed comparatively. Distinguishing features between *C. reflexa* and *Z. jujuba* were identified for each category. Microscopic images and photographs were used to illustrate the observed morphological and anatomical variations.

Results and Discussion Morphological details

The morphological analysis revealed distinct characteristics for *Cuscuta reflexa* and *Ziziphus jujuba*. *Cuscuta reflexa*

exhibits a long, twining, branched, glabrous stem with a pale greenish-yellow color, sometimes dotted with red. Small flowers (2 mm) are borne singly along the stem, and the fruit is a dry, spherical structure with a thin shell containing several small, black seeds. A noteworthy feature is the very bitter taste of the powdered stem.

In contrast, *Ziziphus jujuba* exhibits a shrub or small thorny tree growth habit, reaching up to 15 meters in height. It possesses a deep taproot and numerous drooping branches with tomentose twigs. The leaves are simple, alternate, and ovate with three prominent veins. A key distinguishing feature is the dense, silky hairiness on the underside of the leaves, absent in its close relative, Chinese jujube. The fruit is an ovoid drupe with a fleshy interior that varies in flavor and texture depending on ripeness. The presence of solitary or paired spines at the base of the leaves can also be observed in some *Ziziphus jujuba* specimens.

Anatomical details

Cuscuta reflexa: Microscopic examination of transverse stem sections revealed distinct anatomical features. The epidermis comprised a single layer of thin-walled parenchyma cells. Notably, a well-developed, 7-layered zone of phellogen (cork tissue) was observed underlying the epidermis. The central stellar region displayed a relatively large parenchymatous pith. The vascular system consisted of 8-10 discrete, conjoint, collateral, and open vascular bundles arranged in a ring-like pattern. A distinct starch sheath surrounding the vascular bundles was present, although not remarkably prominent.

Ziziphus jujuba

In contrast to *Cuscuta reflexa*, the stem anatomy of *Ziziphus jujuba* exhibited a more complex organization. The outermost layer consisted of a single layer of large epidermal cells. Stomata were identified as anisocytic type, sunken within the tissue in cross-section. Additionally, papillae (small, finger-like projections) were observed on the abaxial surface of the midrib. The mesophyll tissue exhibited an isobilateral arrangement, featuring 3-4 layers of elongated palisade cells on the adaxial (upper) surface and 2-3 layers of shorter, rounded cells on the abaxial (lower) surface. The vascular system comprised collateral vascular bundles surrounded by parenchymatous bundle sheaths. These observations are consistent with the typical anatomical structure of woody dicotyledonous stems.

Phytochemical screening

Preliminary phytochemical analysis of the ethanolic and aqueous extracts revealed the presence of various secondary metabolites in both *Cuscuta reflexa* and *Ziziphus jujuba*. Qualitative tests identified the presence of flavonoids, alkaloids, tannins, saponins, and terpenoids in *Cuscuta reflexa*. Flavonoids are known for their antioxidant and anti-inflammatory properties, while alkaloids encompass a diverse group with various pharmacological effects. Tannins may play a role in plant defense, and saponins possess potential antimicrobial and anti-inflammatory activities. Terpenoids, a large and diverse class, exhibit a wide range of biological activities including anticancer and anti-inflammatory properties. *Ziziphus jujuba* extracts likely possess a similar range of secondary metabolites, although the specific profiles might differ.

Table 1: Phytochemica	l screening extract	ts of Ziziphus j	<i>ujuba</i> and	Cuscuta reflexa
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Active Components	Ziziphus jujuba		Cuscuta reflexa	
	Ethanol Extract	Aqueous Extract	Ethanol Extract	Aqueous Extract
Phenols	+	+	+	+
Terpenes	+	-	+	+
Steroids	+	+	+	+
Cardiac Glycosides	+	+	+	+
Quinones	-	-	-	-
Plabotannins	-	-	-	+
Saponins	-	+	+	+
Glycosides	-	-	-	-
Alkaloids	+	+	-	-
Tannins	+	+	+	+
Anthraquinones	-	-	+	+

Discussion

The comparative analysis of *Cuscuta reflexa*, a parasitic plant, and its host, *Ziziphus jujuba*, yielded compelling evidence for their distinct characteristics at the morphological, anatomical, and phytochemical levels. These differences highlight the unique adaptations employed by *Cuscuta reflexa* for its parasitic lifestyle (Kaiser, *et al.*, 2015; Rai, *et al.*, 2016)^[11, 18]. The most striking distinction lies in the overall growth form. *Ziziphus jujuba* exhibits a well-developed, self-supporting structure characteristic of a shrub or small tree.

In contrast, *Cuscuta reflexa* lacks roots, leaves, and a robust stem, relying entirely on its host for water, nutrients, and photosynthesis (Kaiser, *et al.*, 2015; Flores-Sánchez & Garza-Ortiz, 2019; Jhu, & Sinha, 2022) ^[11, 6, 10]. This dependence is further reflected in the presence of haustoria (specialized structures for nutrient uptake) not observed in *Ziziphus jujuba*.

Microscopic examination revealed further disparities. *Cuscuta reflexa* possesses a single-layered epidermis, a well-developed cork layer (phellogen), and a large pith. The well-

developed cork layer might be an adaptation to minimize water loss, crucial for a plant lacking roots. The large pith potentially serves for storage, compensating for limited nutrient and water uptake (Sharma, *et al.*, 2010; Khan, *et al.*, 2009) ^[19, 12] Conversely, *Ziziphus jujuba* displays a more complex anatomy with distinct tissues like palisade cells for photosynthesis and a well-developed vascular system for efficient transport (Zarinkamar, 1993; Dinarvand & Zarinkamar, 2006)^[22, 4].

Preliminary screening suggests the presence of various secondary metabolites (flavonoids, alkaloids, tannins, saponins, and terpenoids) in both *Cuscuta reflexa* and *Ziziphus jujuba*. Notably, the specific composition might differ, potentially reflecting their contrasting lifestyles. Further investigation is necessary to identify and quantify these metabolites and explore their potential roles. In *Cuscuta reflexa*, these metabolites could be involved in nutrient acquisition from the host, defense against herbivores, or other aspects of its parasitic strategy (Kaiser, *et al.*, 2015; Landi, *et al.*, 2022; Albert, *et al.*, 2008; Furuhashi, *et al.*, 2011)^[11, 13, 1, 7].



Fig 1: Field photograph of *Casuta reflexa* on *Ziziphus jujuba* ~ 231 ~



Fig 2: The tissues of the stems cross-sections in the species at (10X), where A – Ziziphus jujuba and B – Cuscuta reflexa

Conclusion

The combined analysis of morphology, anatomy, and phytochemistry provides a comprehensive understanding of the distinctions between *Cuscuta reflexa* and *Ziziphus jujuba*. The parasitic nature of *Cuscuta reflexa* is evident in its morphological adaptations and potential reliance on secondary metabolites for survival. Further research focusing on the specific functions of these metabolites and the physiological interaction between parasite and host would offer even deeper insights into this fascinating parasitic relationship.

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