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Research Article

PHARMACOGNOSTIC EVALUATION OF *EUPHORBIA MILII*

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Abstract

Objective: Euphorbia milii is a significant ornamental and medicinal plant found in tropical and subtropical regions. It belongs to the family Euphorbiaceae and the genus Euphorbia. This study was designed to explore the pharmacognostic properties of Euphorbia milii.

Materials and Methods: The pharmacognostic evaluations included organoleptic, microscopic, fluorescence and phytochemical screening. The extraction yield was also studied of n-hexane and methanolic extracts of Euphorbia milii.

Results: Result indicated that methanolic extract of Euphorbia milii has more yield than n-hexane extracts. Powder microscopy showed the presence of scleroids, fibers while phytochemical screening indicated the presence of alkloid, tannins and flavonoids**.**

Conclusion: This study provides the scientific data for the proper identification and establishment of standards for the use of leaves and thorns of *Euphorbia milii*. The phenols and flavonoids detected in the phytochemical analysis might be responsible for medicinal properties of *Euphorbia milii.*

Keywords: *Euphorbia milii*, Extraction , Phytochemical, Flourescence

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INTRODUCTION

The genus *Euphorbia* (family Euphorbiaceae) plants have many useful biological activities such as antiproliferative, antimicrobial, antidiarrheal, anti-inflammatory, antiarthritic, antidiabetic, anti-eczema, antipyretic, and analgesic (Rauf et al., 2013) . *E. milii*, a succulent bush, is used as an ornamental plant in Pakistan and no medicinal use is reported. However, in China it is used to treat abdominal edema and hepatiti. *E. milii* is reported to possess antioxidan, antinociceptiv, and antiproliferative properties. Phytochemical analysis has revealed the presence of terpenoids, flavonoids, fatty alcohols, phytosterols, tannins, and alkaloids in *E. milii*(Fitzgerald et al., 2019).

Antimicrobial resistance (AMR), the resistance in bacteria, viruses, protozoans, and fungi, is a combination of economic and scientific challenges in the development of antimicrobials. On estimation, AMR is the cause of 750,000 deaths globally, which, by the year 2050, may reach to 10 million(Vickers et al., 2001). Antibiotic resistance to bacteria is of particular importance because it can not only be a cause of deadly infectious diseases but can also heavily impact people with chronic conditions such as rheumatoid arthritis, diabetes, and asthma. Crude extracts, being rich in secondary metabolites, can be very promising to overcome the AMR, because they can act in synergy by targeting multiple receptors(Vickers et al., 2001).

The liver is one of the essential organs in our body and regulates the essential body homeostasis processes. Environmental toxins, oxidative stress, microbes, and some medications contribute to the development of hepatic injury(Sofowora et al., 2013). Liver disorders are now among the high priority areas of healthcare. Natural herbal formulations are very appealing in this context and many of them in crude form and isolated pure compounds have been reported to be very effective in the treatment of hepatotoxicity(Rouf et al., 2021).

In the present study, the antibacterial and hepatoprotective effects of *n*-hexane and methanol extracts of leaves and thorns of *E. milii* were evaluated. Additionally, pharmacognostic characters and phytochemical analysis was also done.

MATERIALS AND METHODS

Chemicals and reagents

All drugs and research-grade chemicals were used in this study. The solvents n-hexane and methanol were sourced from Duksan Pure Chemicals.

Collection of plant material and preparation of crude extracts

The leaves and thorns of *E. milii* were collected locally from the premises of Multan, Pakistan and were authenticated by Dr. Sidra Asad (Assistant professor, Department of Botany, The Women University, Multan, Pakistan). A voucher number PFL 294 was issued for future reference. Depending on the experiment performed, plant parts were utilized fresh or were shade dried.

Preparation of crude extracts

The shade dried plant parts were ground coarsely using pestle and mortar and were extracted by maceration. Briefly, 500 grams of leaves and thorns were kept separately in amber colored container in 1500 ml of *n*-hexane, at room temperature for 7 days, with occasional shaking. Then, the material was filtered and the solvent was evaporated by rotary evaporator to obtain crude extract. Maceration

was repeated twice on the same plant material using fresh solvent. The extracts from both macerations were combined and stored at -20°C for future use. The same procedure was repeated with methanol.

Macroscopic characterization of fresh plant parts

The fresh plant parts were characterized by evaluating their macroscopic features such as color, size, odor, inflorescence of flowers, venation pattern of leaves, etc.(Tilburt & Kaptchuk, 2008)

Microscopic analysis of powdered plant parts

Powdered plant parts were evaluated microscopically by putting very small quantity of the powder on glass slide, treating it with chloral hydrate, phloroglucinol, and glycerin, and observing it under the microscope(Leonti & Casu, 2013).

Fluorescence analysis

Fluorescence analysis was performed by treating the powdered plant materials with various solvents and observing them under daylight and UV light. (254 nm and 366 nm).

Phytochemical analysis

Chemical analysis of plant compounds of the crude extracts for the detection of alkaloids, glycosides, tannins, flavonoids, fats and fixed oil, phenol, steroids, and saponins was carried out using standard procedures(van Andel, 2003).

RESULTS

Extraction yield

By maceration, a frequently used extraction technique in our lab, a total of four extracts were prepared. The percent yield (% m/m) of the extracts was calculated as the percentage of the ratio of the weight of crude extract to the weight of dry plant powder (**Table 1**).

Table 1. Yield (% m/m) of prepared extracts

Plant part	**Solent used**	
	***n*-Hexane**	**Methanol**
Leaves	6%	9%
Thorns	3%	6%

Macroscopic characters of collected plant parts

Macroscopic evaluation is often the first step in the identification of plant material. It is mostly achieved by organoleptic evaluation using sense organs and has long been used, not only for identification, but also for quality evaluation of herbal medicinal products.

We evaluated the collected fresh plant parts macroscopically as described below.

Leaves: The dark green colored sessile leaves are obovate in shape, bitter in taste, and possess herbaceous odor. Venation is pinnate and apex is obtuse. Length and width of leaves are 5.0-8.5 cm and 4-5 cm, respectively.

Thorns: The perennial yellowish colored thorns are odorless and bitter in taste. Their size ranges from 2-3cm

Microscopic characters of powdered flowers, leaves, and thorns of *E. milii*

Evaluation of the crude drugs at microscopic levels not only strengthens the macroscopic identification, but in some cases, is the only way to authenticate the plant material e.g., when the drug is present in powdered form. Adulteration by other plant parts can also be easily verified by microscopic analysis.

In this study, we conducted a microscopic evaluation of the powdered plant parts of E. milii. The leaves showed the presence of the lower epidermis, glandular trichomes, and fiber bundles **(Figure 1)**, while the thorns contained starch granules, parenchyma cells, and reticulate cells **(Figure 2)**.

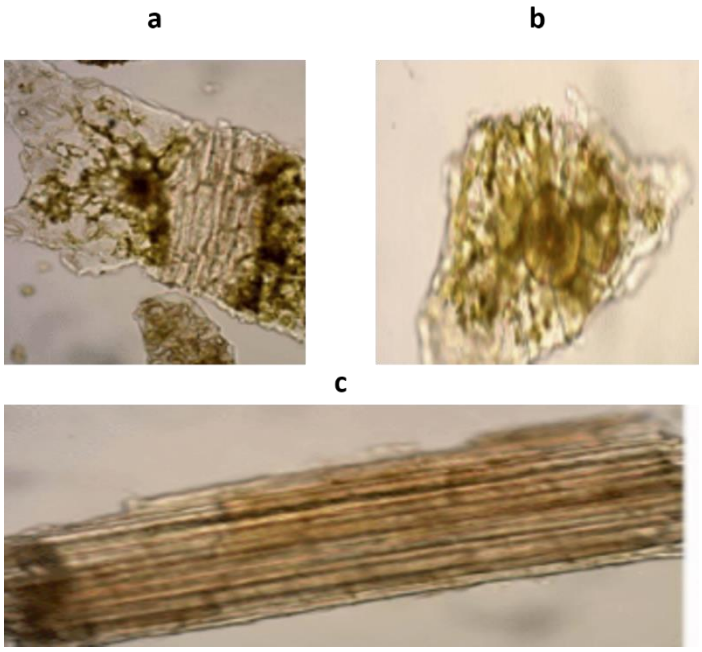

Figure 1. Microscopic structures observed in powdered leaves of *E. milii.* (**A**) Lowerepidermis; (**B**) Glandular trichomes; (**C**) Bundle of fibers.

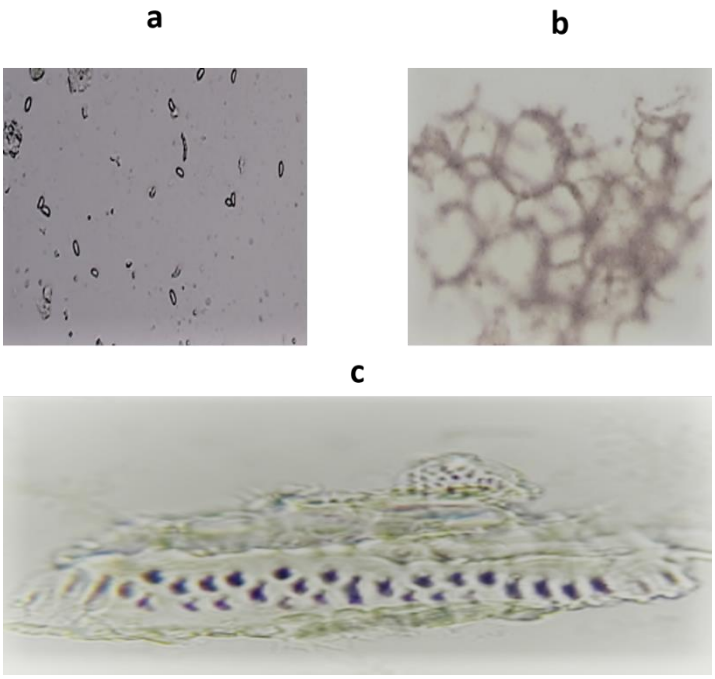

Figure 2. Microscopic structures seen in powdered thorns of *E. milii.* (**A**) Starch granules; (**B**) Parenchyma cells; (**C**) Reticulate cell

Fluorescence analysis

Fluorescence is a phenomenon in which different phytoconstituents exhibit specific color response when they react with specific solvents. Hence, fluorescence analysis is a very effective quality assessment parameter of crude drugs(Rasool Hassan, 2012). We did fluorescence analysis of our powdered plant parts by treating them with various solvents and observing them under day light and UV (**Table 2**).

Table 2. Fluorescence analysis leaves and thorns of *E. milii* in powdered form

Reagent	Ultraviolet light				Day light	
	Leaves		Thorns		Leaves	Thorns
	254 nm	366 nm	254 nm	366 nm		
E. milii + Acetic Acid	Green	White	Yellow	Brown	Brown	Brown
E. milii r+ 10% HCl	Black	Green	Green	Orange	Brown	Green
E. milii + Methanol	Black	Green	Purple	Brown	Brown	Green
E. milii + Sulphuric Acid	Green	Green	Brown	Brown	Yellow	Black
E. milii + Acetone	Black	Black	Purple	Orange	Orange	Green
E. milii + 10% Ferric Chloride	Black	Green	Brown	Orange	Red	Green
E. milii + Distilled Water	Black	Green	Brown	Brown	Brown	Brown
E. milii + 50% Nitric Acid	Green	Green	Green	Brown	Brown	Orange
E. milii + 10% Ethanol	Green	Green	Brown	Orange	Brown	Brown
E. milii + 99% Ethanol	Green	Green	Green	Brown	Brown	Green
E. milii + Sodium Hydroxide	Green	Green	Purple	Brown	Yellow	Brown
E. milii + Chloroform	Brown	Black	Green	Orange	Brown	Green
E. milii + Benzene	Brown	Green	White	Brown	Brown	Green
E. milii + Acetonitrile	Brown	Black	Brown	Orange	Brown	Green
E. milii + Picric Acid	Yellow	Brown	Yellow	White	Black	Yellow
E. milii + Diethyl Ether	Orange	Black	Black	Orange	Orange	Brown
E. milii + *n*-Butanol	Brown	Black	Green	Brown	Green	Green

Phytochemical evaluation

To explore the chemical constituents of the crude extracts, we started analyzing the classes of phytochemicals present in these crude extracts. Some classes of phytochemicals are well-known for certain biological activities and toxicity. Different classes of phytochemicals detected in in leaves and thorns of *E. milii* are summarized in **Table 3**.

Table 3. Detection of different classes of secondary metabolites leaves, and thorns of *E. milii*.

Phytochemical test	Observation		Results	
	Leaves	**Thorns**	**Leaves**	**Thorns**
1. Test for alkaloids				
Dragendorff's	Orange ppt	Red soln.	+	−
Mayer's	Off white ppt	Red ppt	+	−
Wagner's	Reddish ppt	Yellow soln.	+	−
Hager's	Yellow ppt	Green soln.	+	−
2. Test for glycosides				
Bromine water	Pale yellow color	Reddish soln.	−	−
Molisch	Turbid soln.	Redsdish soln.	−	−
Conc sulfuric acid	Green ppt	Reddish ppt	−	−
3. Test for tannins				
Ferric chloride	Blackish ppt	Blackish ppt	+	+
Gelatin	Off White ppt	Off White ppt	+	+
Alkaline reagent	Reddish soln.	Reddish ppt	+	+
4. Test for flavonoids				
Alkaline reagent	Redish ppt	Redish ppt	+	+
Ferric chloride	Blackish ppt	Blackish ppt	+	+
5. Test for fats and fixed oil				
Stain test	Light green stain	Dark green stain	-	+
6. Test for phenols				
Phenol test	Blackish color	Blackish color	+	+
7. Test for steroids				
Salkowski test	No ring on junction	No ring on junction	+	+
8. Test for saponins				
Frothing test	No froth formed	No froth formed	−	−

+: detected; −: not detected; ppt: precipitates; soln.: solution.

DISCUSSION

Extractive value serves as a valuable tool for assessing the composition of phytoconstituents within plant materials. These values not only validate the presence of specific constituents but also quantify the ones soluble in particular solvents (Rauf et al., 2013). Similarly, the percentage yield of leaves is 6% in n-hexane and 5% in methanol (Table 1). For thorns, the yields are 3% in n-hexane and 2.5% in methanol (Table 1). These results indicate that n-hexane extracts contain a higher concentration of constituents compared to methanol. Powder microscopy serves as an indicative tool for further assessing pharmacological activities (Rasool Hassan, 2012). The leaves show glandular trichomes, the lower epidermis, and fiber bundles (Fig. 1). The thorns of Euphorbia milii reveal reticulate cells, starch granules, and parenchyma cells (Fig. 2). Phytochemical analysis is essential for identifying the constituents in plants, which are then isolated and contribute to the plant's various pharmacological activities. Similarly, the methanolic extract of the aerial parts of Euphorbia milii (leaves and thorns)

contains flavonoids, tannins, alkaloids, phenols, fats, and fixed oils. The details of these secondary metabolites are summarized in Table 3. Euphorbia milii contains flavonoids, which are primarily polyphenolic compounds found in plants as either aglycone or glycone derivatives. These flavonoids are crucial for human health because their polyphenolic nature provides anti-inflammatory effects. (Reddy et al., 2017) anti-carcinogenic, anti-diabetic, anti-viral (Leonti & Casu, 2013), They also possess liver protective, spasm reducing, diuretic, hypotensive, immune-stimulating, and anti-mutagenic properties. Plants rich in polyphenols function as effective antioxidants, capable of scavenging superoxide radicals and inhibiting lipid peroxidation. Furthermore, flavonoids may act as selective inhibitors of cyclooxygenase. (COX-2).(Fitzgerald et al., 2019). Fluorescence is a notable phenomenon that exhibits various colors due to the presence of specific compounds in plant material. Each phytocompound produces a distinct fluorescent color (Rauf et al., 2013). Non-fluorescent compounds may fluoresce when mixed with fluorescent impurities. The fluorescent technique is extremely sensitive and allows for the accurate analysis of pharmaceutical samples. In this study, powdered parts of Euphorbia milii displayed a variety of colors under both visible and UV light after being treated with different acids, alkalis, and reagents. Analyzing the brightness of these powders offers valuable information on possible adulteration, making it a useful tool for detecting tampering. This approach is crucial before conducting a comprehensive phytochemical investigation.

CONCLUSION

This research offers scientific evidence to facilitate the accurate identification and formulation of standards regarding the utilization of *Euphorbia milii* leaves and thorns. The presence of phenols and flavonoids, as revealed in the phytochemical analysis, could potentially account for the medicinal properties attributed to *Euphorbia milii.*

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CONFLICT OF INTEREST

The author stated that there is no conflict of interest.

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