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Microscopic Evaluation and Qualitative Phytochemical Screening of *Corchorus olitorius* L. (Molokhia) Leaves

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Abstract

Corchrous is a genus which belongs to Tiliaceae family distributed across Asia and Africa. In the world, the *Corchorus* genus encompasses 75 taxa. Widely distributed across the tropics, *Corchorus olitorius* L. (Molokhia) is most likely found in every tropical African nation. Its use as a wild or farmed vegetable has been reported by numerous countries in tropical Africa. In Cyprus, this plant is used to prepare a dish after drying in the summer. The aim of this study was to investigate microscopic evaluation and phytochemical profile of the leaf extracts of *C. olitorius*. In microscopic evaluation experiments, powdered leaf part of the plant was examined under the microscope. For determination of phytochemical profile, some of secondary metabolites were determined qualitatively by chemical reactions. Microscopic analysis revealed that molokhia leaves displayed epidermis, stoma, calcium oxalate crystals, spongy parenchyma, glandular hair, trichome, midrib. The phytochemical profile identification of the leaf extracts of *C. olitorius*, revealed the presence of some active ingredients such as carbohydrates, cardioactives, and flavonoids.

Keywords

Corchorus olitorius, leaf, microscopy, plant tissue, phytochemistry.

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INTRODUCTION

Corchorus olitorius L., also known as molokhia, Egyptian spinach, Nalta jute, or Tossa jute, is a member of the Tiliacea family (Table 1). The annual plant *C. olitorius* that can reach a height up to 2-4 meters and is stiff and fibrous (Islam, 2013). Its border leaves are sharply indented and alternate. *C. olitorius* produces tiny yellow blooms with five petals that eventually develop into a brown, multiseeded pod (Loumerem et al., 2016). It is spread by seeds and can be grown in household gardens or tolerated as a wild vegetable in crop fields (Begum et al., 2011).

Table 1: Taxonomy of *C. olitorius* L. (Islam, 2013).

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Malvales
Family	Tiliaceae – Linden family
Genus	*Corchorus* L. – corchorus
Species	*Corchorus olitorius* L. – nalta jute
	Corchorus capsularis L. – white jute

This dark green leafy vegetable is widely consumed as food in the Middle East, Eastern Mediterranean, and Cyprus. Young leaves are tasty and soft, however older leaves become woody and fibrous, rendering them less suitable for consumption (Soykut et al., 2018). Known as a popular leafy soup, it is prevalent in all tropical and sub-tropical climates (Kundu et al., 2012). It is considered a nutrient-dense vegetable because of its high concentration of vitamins and other nutraceutics such as vitamin B1, B2, A, C, E, folic acid, minerals, *beta*-carotene, calcium, and iron. High levels of phenolic components, such as quercetin and caffeoylquinic acids, suggest that it may possess antioxidant qualities and be linked to protection against chronic illnesses such as diabetes, cancer, heart disease, and hypertension (Giro et al., 2016). *C. olitorius* is used to treat ulcers, heart failure, female infertility, typhoid fever, and malaria in West Africa (Nyadanu et al., 2017). In addition to its antidiabetic actions (Airaodion et al., 2019), the vegetable was reported to possess antibacterial (Ilhan et al., 2007), anti-inflammatory (Handoussa et al., 2013), antiobesity (Wang et al., 2011), and gastroprotective qualities (Nakaziba et al., 2020). Seeds of *C. olitorius*, have been used as demulcents, diuretics, and purgatives, as well as in cases of chronic cystitis. The seeds can also be used against heart failure because they contain cardenolides, also known as cardiac glycosides (Al-Yousef et al., 2017). The leaves have been used to treat tumors, colds, fevers, constipation, gonorrhea, and chronic cystitis (Islam, 2013, Zakaria et al., 2006). Although other parts can also be used the

leaf is the most frequently used part of the plant in folk medicine. The parts used in traditional medicine and the areas of use of these parts are given in Table 2.

Table 2: Usage of *C. olitorius* L. in traditional medicine (Adebo et al., 2018).

Usages	Parts of the plant used
Malaria	Whole plants, leaves, roots, leafy stems
Typhoid fever	Leafy stems, flowers, roots, leaves, whole plant
Fever	Leafy stems, flowers, leaves, whole plant
Female fertility	Whole plant
Diarrhea	Leaves
Ulcera, Colic	Leafy stems, leaves, leaves and seeds, whole plant
Itera	Leaves, leafy stems
Heart failure	Whole plant
Child malnutrition	Leaves
Sexual weakness	Roots

C. olitorius contain many different secondary metabolites such as phenolic acids, flavonoids and some steroidal compounds. The leaves and the flower parts of the plant are rich in hydroxycinnamic acid derivatives such as caffeic, coumaric, rosmarinic and ferulic acid. Flavonoid derivatives (luteolin, apigenin, quercetin, kaempferol, rutin, and naringenin) were identified in leaves and flowers. In addition, steroidal compounds such as stigmasterol, β-sitosterol, canarigenin, fusudic acid, and campestrol were detected in the seed of the plant (Abdel-Razek et al., 2022).

MATERIALS AND METHODS

The dried leaves of *C. olitorius* L. were purchased from a market in Famagusta, Cyprus in September 2024. The plant was identified using Flora of Cyprus (Meikle, 1977). Microscopic materials and chemical reagents were supplied from Eastern Mediterranean University Laboratories.

Microscopic evaluation of *C. olitorius* L. leaves

For the microscopic studies, leaf sample was fine powdered and mounted in chloralhydrate reagent. Microscope slide was heated with Bunsen burner for fixation of the sample and the microscopical characteristics of leaf powder were investigated.

Phytochemical screening of *C. olitorius* L. leaves

Test for carbohydrates

After 7 gr of the plant sample was weighed and crushed using mortar and pestle, it was transferred to the beaker. 40 mL of distilled water was added to the crushed plant sample in the beaker and stirred for 5 minutes. The aqueous part of the suspension is filtered off. 3.5 mL of 10% lead acetate solution was added to the filtrate drop by drop followed by filtration of the solution. Through this process, compounds such as chlorophyll, flavonoids, and tannins are

precipitated and separated from the aqueous extracts. 4 mL of 2.5% disodium hydrogen phosphate was added dropwise to the filtrate, and then the solution was filtered. Extract was used for the determination tests.

Fehling test: 1 mL of the extract prepared as mentioned above is replaced in a test tube. 2 mL of Fehling A and then 2 mL of Fehling B were added to the extract. The solution was heated using bunsen burner. Formation of red colored Cu_2O shows precipitation that indicates the presence of a carbohydrates.

Molisch test: To 1 mL of the extract, 5 drops of 5% alcoholic α-naphthol solution was added in a test tube. The test tube was slightly tilted and concentrated sulphuric acid was leaked down into the tube, where formation of violet-purple ring proves the presence of a carbohydrate.

Seliwanoff test: 2.5 mL of Seliwanoff reagent was added to 1 mL of the extract in a test tube. Then, the solution was subjected to boil. Formation of red color indicates the presence of a ketose in the solution. Aldoses give late reaction results, and their presence produces light red color. On the other hand, saccharides such as pentoses lead the formation blue-green color.

Test for flavonoids

2% decoction was prepared from the powdered plant sample that is dissolved in 15 mL of 50% ethanol solution.

Ferric chloride test: 2-3 drops of 5% $FeCl_3$ solution in water were added to 3 mL of the extract. Green and blue-black colour indicates the presence of flavonoids.

Sodium hydroxide test: 2-3 drops of 10% NaOH solution were added to 3 mL of the extract. Bright yellow color indicates the presence of flavonoids.

Sulphuric acid test: 2-3 drops of sulphuric acid solution were added to 3 mL of the extract. Red color formation indicates the presence of flavonoids.

Cyanidin (Shinoda-Shibata) test: 0.5 mL of concentrated hydrochloric acid and magnesium or zinc dust at the tip of spatula were added to the filtrate, where hydrogen gas release is observed by bubbles causing an orange color for flavones, a red color for flavonols, and a purple color for flavanones.

Test for cardioactive glycosides

1 gr of the powdered plant sample was boiled for 3 minutes with 25 mL of 50% ethanol in a water bath and then filtered off. Into the filtrate, 5 mL of 10% lead acetate was added where precipitation was observed. Precipitation was filtered off and the filtrate was hydrolysed by heating with diluted sulphuric acid for 3 minutes. 1 mL of the solution was taken into a test tube for Keller-Kiliani reaction and the rest was mixed with chloroform in a separating funnel. The organic chloroform layer (bottom layer) was taken and used in Baljet test.

Baljet's test: 5 mL of the dissolved extract was evaporated in a capsule. 1 mL of Baljet reagent and 5 drops of 6% sodium hydroxide were added to the test tube. Formation of an orange color displays the presence of a cardenolides.

Keller-Kiliani reaction: To a small amount of solution separated in a test tube, sulfuric acid was leaked from edge of the tube, where two layers were formed. Formation of a brown-red ring between the two layers shows the presence of 2-deoxy sugars.

Test for saponins

Foam test: 0.5 gr of the powdered plant was placed in a test tube with 10 mL hot water. After cooled down, it was shaken vigorously for approximately 10 seconds. In the presence of saponin, a foam layer of 1-10 cm in height forms, which remains stable for at least 10 minutes and does not disappear with the addition of 1-2 drops of 2 N HCl.

RESULTS

Microscopic evaluation of *C. olitorius* leaves

Powdered leaf sample was mounted in chloral hydrate reagent. After investigation under microscope; epidermis, stoma, calcium oxalate crystals, spongy parenchyma, glandular hair, trichome, and midrib elements were detected (Figure 1).

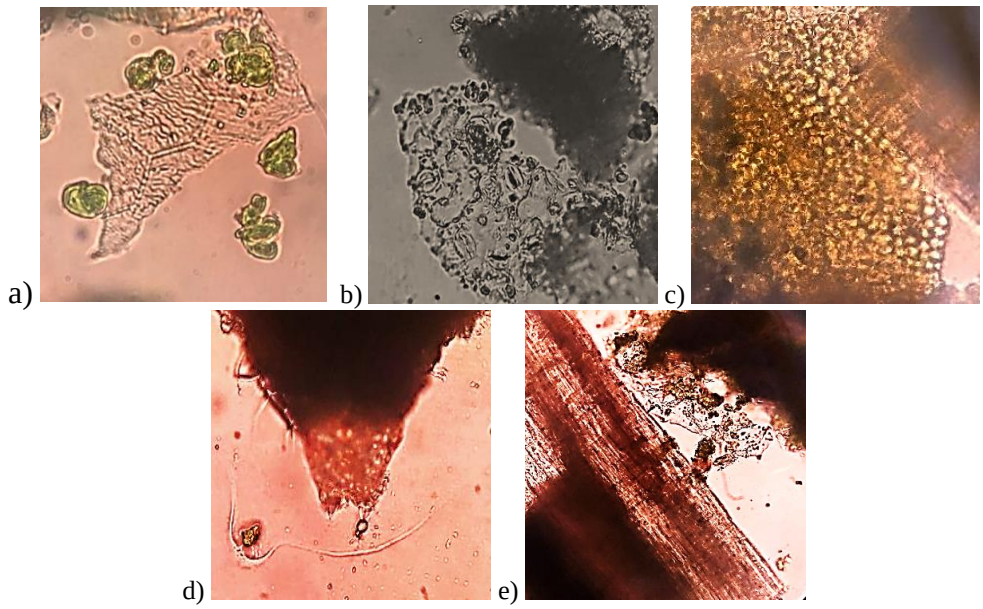

Figure 1: Microscopic images of *C. olitorius* leaves.
a: epidermis, b: stoma and calcium oxalate crystals, c: spongy parenchyma, d: glandular hair and trichome, e: midrib.

Phytochemical screening of *C. olitorius* L. leaves

In the present study, phytochemical screening was crucial for discovering novel sources of chemicals with therapeutic and industrial use that have been chemically studied in medicinal

plants. Primary and secondary metabolites were qualitatively analysed. The phytochemical profile revealed the presence of some active ingredients such as carbohydrates, cardioactive glycosides, and flavonoids (Table 3).

Table 3: Qualitative phytochemical screening of *C. olitorius* leaves.

Primary/Secondary metabolites	Results	Name of tests
Carbohydrates	+	Fehling test, molisch test, seliwanoff test
Flavonoids	+	Ferric chloride test, sodium hydroxide test, sulphuric acid test
Cardioactive glycosides	+	Baljet's test, Keller-Kiliani test
Saponins	-	Foam test

DISCUSSION

In a previous study, microscopic evaluation of *C. olitorius* showed the presence of epidermis, stomata, glandular hair, and trichome (Varban et al., 2021). In another study, the surface view, transverse section and the powder of *C. olitorius* leaves were analysed under microscope and the presence of midrib non-glandular trichomes, epidermis, palisade parenchyma, spongy parenchyma, stoma and phloem were observed (Khan et al., 2022). As a results of the examination of the leaf of *C. capsularis* under the microscope, the presence of epidermis, stomata, calcium oxalate crystals, spongy parenchyma, trichome, and midrib similar to *C. olitorius* except glandular hair was detected (Mallesh et al., 2023).

Phytochemical characteristics of *C. olitorius* were investigated by numerous studies. In one of the studies, it was found that fresh *C. olitorius* leaves consisted of steroids, cholesterol, alkaloids, phenols, flavonoids, riboflavin, saponins, and terpenoids (Sadat et al., 2017). The ethanolic extract of dried *C. olitorius* leaf revealed the presence of tannins, steroids, saponin, terpenoids, cardiac glycosides, and alkaloids as chemical constituents (Ujah et al., 2014). Although microscopic and phytochemical studies conducted on this species are present, studies conducted on the species grown in Cyprus are limited.

Microscopic evaluation and phytochemical analysis are very important to identify plant species. Further studies are needed to characterize *C. olitorius* species in detail.

CONCLUSION

In conclusion, the present study will provide a background for the characteristics and phytochemistry of *C. olitorius* leaf. Further pharmacognostic analysis of the leaf will offer specific criteria for accurate identification. Phytochemical and instrumental analyses alongside biological activity studies related to the plant's traditional uses are among future directions.

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