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REVIEW ARTICLE

Olea dioica Roxb.: An Underexplored Medicinal Plant with Promising Biological Activities and Health Benefits – A Comprehensive Review

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ABSTRACT

The plants are a valuable source of possible assemblies for the creation of novel chemotherapeutic drugs. During ancient times, medicinal plants were used to flavor and preserve food, as well as to cure health issues and prevent epidemic diseases. *Olea dioica* Roxb., belongs to the family Oleaceae. It is a vital medicinal tree used by local *Siddha* tribes. Different parts of the plant *O. dioica* viz., leaves, bark, root, and fruits are consumed as traditional medicine to cure fever, cancer, snake bite, rheumatism, skin diseases and many more. While using standard protocols, different extracts of plant parts have shown the presence of micro and macro-elements, carbohydrates, flavonoids, fats, steroids, phenolic compounds, proteins, saponins, reducing sugars and alkaloids. GC-MS analysis results exhibit hexadecanal, octadecanal, 6-octadecenoic acid, lupeol trifluroacetate, 12-oleanen-3-yl acetate (3Alpha), urs-12-en-3-ol, acetate and lu-20(29)-en-3-ol, acetate, (3.beta.). Ethanolic extract of the bark of *O. dioica* was found to have a pure compound benzene ethanol, 4-hydroxy-alcohol which has a good scavenging activity.

Keywords: *Olea dioica*; Phytoconstituents; Aphrodisiac; Analgesic; Anticancer; Anti-Alzheimer's

INTRODUCTION

The Western Ghats, whose evergreen and semi-evergreen forests have high levels of tree variety and endemism, for example, *Terminalia paniculata, Aporusa lindleyana, Olea dioica*, *Mesua ferrea, Vateria indica, Elaeocarpus tuberculatus, Celtis timorensis, Hopea parviflora, Lagerstroemia macrocarpa, Holigarna arnottiana, Hydnocarpus laurina, Memcylon umbellatum, Pavetta crassicaulis, Pavetta indica*, and *Careya arborea* [1]. India is one of the countries having a rich heritage of traditional medical structures as well as a diverse biodiversity to supplement the herbal demands of treatments with these traditional medical systems. *Ayurveda, Siddha*, and *Unani* are three recognized Indian medical systems that incorporate herbs and minerals in their formulations [2].

Olea dioica is regarded as an underexplored and underutilized species despite its acclaimed medicinal potential and splendid ethnobotanical history. Latest

research has confirmed numerous traditional uses, but further exploration is necessary to fully recognize its therapeutic value. The complete chloroplast genome sequence of *Olea dioica* Roxb., (Oleaceae) in June 2024 indicates it is still in the initial stages of scientific characterization [3]. Multiple floras and herbaria list its conservation status as "Not Evaluated" (NE) by the IUCN, suggesting that not enough data is available to assess its risk of extinction [4].

Despite being underexplored, of late studies emphasize its favourable qualities and potential for future research. Chemical analysis has ascertained various bioactive compounds in its leaves with potential applications, including esters of fatty acids and squalene. Research has highlighted the plant's significant antioxidant activity [5]. Furthermore, leaf extracts demonstrate a concentration-dependent ability to inhibit the growth of human cancer cell lines (colon and breast carcinoma), pointing towards potential anticancer applications [6]. Methanolic leaf extracts have shown effectiveness against a broad spectrum of pathogenic bacteria and fungi. This suggests potential for developing new antimicrobial therapies [7]. Studies have provided scientific backing for some of its traditional medicinal uses. For example, the use of its fruit to boost zinc and magnesium levels aligns with findings showing these minerals are present in high quantities [8]. The recent sequencing of its chloroplast genome provides a valuable genetic baseline for future phylogeographic and population genetic studies [3]. In conclusion, while *Olea dioica* is not completely unknown, it remains a leading example of an underexplored medicinal plant. Its documented traditional uses are now being supported by preliminary scientific research, but it still lacks complete studies needed to fully reveal its capability for modern medicine and justifiable utilization.

Experimental plant

Olea dioica Roxb. Scientific classification
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Oleaceae
Genus: *Olea*
Species: *dioica* Roxb.

Fig. 1: *Olea dioica* Roxb., leaves

Description and distribution

Plant diversity and endemism are high in the Western Ghats' evergreen forests. The Oleaceae family includes *O. dioica* Roxb., a significant folkloric ethnomedicinal plant. These trees up to 15 meters tall grow in open evergreen to semi-evergreen forests and wet deciduous woods up to 1200 meters altitude in India's Western Ghats. The tree bark is brownish, rough, with a pale brown blaze. The branchlets of young trees are sub-quadrangular, lenticellate, and glabrous. Leaves are simple, opposite, decussate Fig. 1; petiole 0.6-1.3 cm long, canaliculate; lamina 7.5-17.5×2.3- 7.5 cm, elliptic to elliptic-oblong, apex gradually acuminate to sub-acute, base acute or attenuate, margin distantly serrate (with strong teeth) or entire, coriaceous to sub-coriaceous, glabrous; midrib flat above, usually reddish when dry [9]. Inflorescence is axillary divaricate panicles; flowers are cream-white, polygamo-dioecious and it has 0.4 cm long pedicel. When ripe, the fruit is drupe-like, ellipsoid, and blue; it has only one seed. The plant's roots have therapeutic qualities and are used in *Siddha* medicine as remedy for snake bites and cancer. The fruits of *O. dioica* Roxb. were utilized by tribes in Maharashtra to cure skin diseases. In rheumatism, the bark and fruit paste are utilized; the bark decoction is used to cleanse old wounds and to treat fever. Tribes in Kerala's jungle have historically utilized ripe fruits [10]. A substantial activity of antibacterial and antifungal properties has been shown in methanol extract [11].

Vernacular names

Assamese: *Bon-bholuka, Poreng*
Kannada: *Bilisarali, Edale, Kalluthodi, Tadale*
Malayalam: *Edala, Etala, Irippa, Palarana, Vetila*
Marathi: *Parjamb*
Tamil: *Idalai koli, Payar, Yedalei*

Parts used

Flowers, seeds, bark, leaves and fruits.

Physico-chemical parameters and nutritive value

The selected plant was analysed for the physico-chemical parameters for example pH, alcohol soluble extract,

foreign matter, water soluble extract, total ash content, water soluble ash and acid insoluble ash were determined. Macro-elements like, crude carbohydrate, crude fat, crude protein, macronutrients- sodium, potassium and phosphorus; micronutrients- magnesium, copper, lead, cadmium, manganese, zinc and calcium were analyzed using standard techniques [12].

Physicochemical properties are necessary to check plant materials or purity of the sample. The percentages of ash originate in stem and root were maximum because; the maximum mass of the tree is occupied by stem and root. The concentration of hydrogen ions is directly proportional to pH. Each part of the plant *O. dioica* shows different physico-chemical values. The alkaline pH of the root, stem, leaf, fruit and flower exhibit neutral pH. The study says it is because of water translocation from root to flower [12].

Nutrients

Macronutrient analysis of *O. dioica* has shown that, stem and root contain high calcium (Ca), potassium (K), phosphorus. The major constituent of DNA, RNA and amino acids called nitrogen was also highly distributed in stems and roots. Magnesium is present more in fruits and leaves, sodium was found more in flowers and leaves.

Further, copper and iron were mainly distributed in fruits and leaves. In stem and root manganese were noticed. Zinc was present in an appreciable amount in fruits and leaves of *O. dioica*. Heavy metal cadmium was also found to be present in the highest level in leaf and stem, but the edible fruit possesses less cadmium (Cd) and absence of lead (Pb). Precisely, the maximum nutritive value of micro and macronutrients are present mainly in leaf, stem, fruit and root [12].

The Jenway-PFP-7 FPM Compressor Unit-122 Flame Photometer is being used to analyse sodium and potassium microelements. Phosphorus was examined by the Spectrophotometer Jenway 6300. Using atomic absorption spectra GBC 932 AA/AAS, the microelements calcium, magnesium, zinc, copper, manganese, iron, lead, and cadmium were examined. The standard method 15 was used to determine the nutritional value.

Phytochemical screening

O. dioica Roxb. belonging to the family Oleaceae, is a vital medicinal tree used by local *Siddha* tribes. Different parts of the plant viz., leaves, bark, root, and fruits are treated as traditional medicine to cure fever, cancer, snake bite, rheumatism, skin diseases and many more. Phytochemical screening of different extracts from different parts of *O. dioica* was accomplished to determine the occurrence of carbohydrates, flavonoids, fats, steroids, phenolic compounds, proteins, saponins, reducing sugars and alkaloids using standard protocols Table. 1.

Three different solvent extracts viz., methanol, petroleum ether, chloroform [2, 10] and ethanol [2, 12-14] were screened for phytochemicals. Methanol extract of bark of *O. dioica* indicates the presence of steroids, glycosides, saponins, flavonoids, and phenols, chloroform extract showed the existence of tannins and flavonoids, but the petroleum ether extract contains no phytochemical compounds comparatively [11].

Table 1: Phytochemical analysis of *O. dioica*

Secondary metabolites	Extracts			
	Petroleum ether	Chloroform	Methanol	Ethanol
Alkaloids	-	-	-	+
Saponins	-	-	+	-
Tannins	-	+	+	-
Flavonoids	-	+	+	+
Steroids	-	-	+	-
Glycosides	-	-	+	-
Phenols	-	-	+	+
Steroids	-	-	+	+

Vimalkumar *et al.,* have performed a comparative study between ethanol extracts of both fungus infected and non-infected *O. dioica* leaves. Both the extracts contain similar phytoconstituents except carbohydrates [12, 15]. Analysis of total phenolic and flavonoid content of methanol extract of *O. dioica* flowers have shown the highest value [13].

The crude extract of *O. dioica* leaves were subjected to column chromatography and collected about 15 fractions which were eluted at a flow rate 3ml/min. The components of the F_2 fraction were checked using GC-MS among the 15 fractions. The results of GC-MS found Hexa decanal, Octa decanal, 6-Octadecenoic acid, Lupeol trifluoroacetate, 12-Oleanen-3-yl acetate (3Alpha), Urs-12-en-3-ol, acetate and Lu-20(1)-en-3-ol, acetate, (3.beta.) [14]. Ethanolic bark extract of *O. dioica* was found to have a pure compound benzene ethanol, 4-hydroxy-alcohol which have good scavenging activity [9].

Scientific studies have recognised several active compounds Table. 2 and minerals in different parts of the plant, including leaves, bark and fruit [10, 11, 13].

Table 2: Key phytochemicals and their health benefits

S. No.	Phytochemical	Associated health benefit	How it works
1.	Flavonoids	Antioxidant, antimicrobial, cytotoxic (anticancer) activity	These phenolic compounds protect against oxidative stress by scavenging free radicals. This activity helps reduce cellular damage and inflammation, which are contributing factors to chronic diseases and aging.
2.	Phenols	Antioxidant, antimicrobial activity	Studies show that a higher phenolic content correlates with increased antioxidant and antimicrobial effects. A methanolic extract of the leaves, rich in phenolic content, exhibited significant antioxidant activity.
3.	Saponins	Antimicrobial activity	Extracts from the bark and leaves of *O. dioica* contain saponins, which have been shown to have antimicrobial properties against various bacterial strains.
4.	Steroids & Sterols	Antimicrobial activity	These compounds are also present in methanolic extracts of the plant's bark and contribute to its antimicrobial effects.
5.	Glycosides	Antimicrobial activity	Glycosides in the bark extract further contribute to the plant's effectiveness against pathogenic bacteria.
6.	Benzene ethanol,4-hydroxy-alcohol	Antioxidant and cytotoxic activity.	This isolated compound, extracted from the bark, has demonstrated potent antioxidant and cytotoxic effects in laboratory studies. Its cytotoxic activity against cancer cells is comparable to the standard drug curcumin.

Table 3: Mineral contributions and their support in body functions

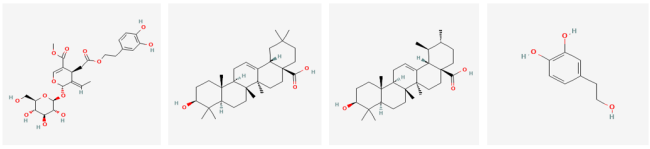
S. No.	Minerals	Associated support to body functions	How it works
		Macronutrients	
1.	Calcium (Ca)	Cell signalling	Calcium is found in stem and root of *Olea*. Calcium has a vital role in both plants and animal metabolism, acts as ion gated channels in cell signalling function.
2.	Sodium (Na)	Maintenance of blood pressure	Sodium is found in flower and leaf of *Olea*. In the human body sodium has a vital role in maintaining the blood pressure, balance the body fluid, normal functioning of nerve and muscles.
3.	Magnesium (Mg)	Blood sugar regulation	The fruit is a source of magnesium, which is vital for maintaining bone stability, and ensuring normal nerve and muscle function.
4.	Potassium (K)	Maintenance of body fluid	Potassium is found in stem and roots of *Olea*. Potassium is a vital macronutrient which helps in the maintenance of body fluid, in the contraction of muscles, neural stability, important enzyme activation, maintenance of proper blood pressure and proper formation of skeletal bones.
5.	Phosphorus (P)	Energy metabolism	Phosphorus is found in root and leaves of *Olea*. Phosphorus plays an important role in energy metabolism and bone mineralization; phosphorus take part in the structural component of DNA and RNA.
6.	Nitrogen (N)	Major constituent of Nucleic acids	Nitrogen is found in stem and root of *Olea*. It is a major constituent of DNA, RNA, amino acids. Nitrogen is required in the proper maintenance of normal growth of cell and proper functioning of muscles. Nitrogen has a vital role in protein synthesis, enzyme synthesis. In the cell cycle, nitrogen has an important role.
		Micronutrients	
1.	Manganese (Mn)	Antioxidant property	Manganese is found in higher concentrations in the roots and stems, suppress reactive oxygen species. It is a cofactor for enzymes involved in metabolism and is crucial for the proper function of thyroid and sex hormones, maintenance of blood sugar levels, and blood clotting.
2.	Zinc (Zn)	Growth hormone synthesis, activation of T lymphocytes	Zinc is present in the fruits and leaves. Zinc supports the proper maintenance of neurophysiological function. It is also known to help with skin protection.

S. No.	Minerals	Associated support to body functions	How it works
3.	Iron (Fe)	Vital component of RBCs	In *O. dioica* plant parts iron (Fe) was majorly found in fruit and leaf. In the human body, iron is a vital component of red blood cells (RBC), constituent for many proteins, enzymes and hormones, the function of haemoglobin and myoglobin, a cofactor for enzyme and in electron transport.

Important mineral contributions

The leaves and fruits of *Olea dioica* contain appreciable amounts of essential minerals [16, 17] that support normal body functions Table. 3.

Chemical structures of some major identified phytochemicals of *Olea dioica* (Source: PubChem)



Oleuropein Oleanolic acid Ursolic acid Hydroxytyrosol

Biological activities

The combined action of the nutrients and phytochemicals underlies the traditional and scientifically validated health benefits of *Olea dioica* such as analgesic, antioxidant, antidiabetic, antifungal, antimicrobial, cytotoxic, anti-inflammatory, skin health, neuroprotective and aphrodisiac activities.

Analgesic activity

Analgesics are pain relievers that act on the central nervous system (CNS) and peripheral pain mediators without altering consciousness. The analgesic activity was carried out to determine the acute toxicity by administering single oral dose of 2000 mg/kg body weight to Swiss albino mice which resulted no change in general animal behavior and mortality. The analgesic effect of extract was examined by tail flick method in Swiss albino mice [15]. Different concentrations of extract were administered to the animals and pentazocine was used as a positive control. Significant analgesic activity was noticed in extract and positive control. The extract has an increased pain threshold, indicating that it has analgesic properties [18].

Antioxidant activity

The methanolic extract of *O. dioica* flower was examined to check the free radical scavenging ability by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and ferric-reducing antioxidant power (FRAP) assay. Ascorbic acid was used as the standard. The results of both the assays have shown that antioxidant activity were lesser than the ascorbic acid [13]. The bark and leaf extracts were extracted using the solvents hexane, petroleum ether, chloroform, ethanol and water. Among all the extracts, ethanol extract has shown appreciable radical scavenging activity (DPPH and ABTS assays) and the aqueous extract of bark and leaf have shown moderate scavenging activity (NBT and hydroxyl scavenging method). The ethanol crude extract has also revealed excellent metal chelating activity. The scavenging activity of all the assays were expressed in terms of % inhibition and IC_{50} values (µg/ml) [9].

Antidiabetic property

Notably, *O. europaea* bud extracts were found to suppress pancreatic lipase and α-amylase activity in several Italian cultivars by Perri *et al.,* [20] With IC_{50} values of 33.21 ± 0.23 µg/mL for α-amylase and 1.27 ± 0.04 mg/mL for lipase inhibition, the investigation discovered a considerable reduction of digestive enzymes. Akhtar *et al.,* [21] investigated the antioxidant properties and potential as antidiabetic agents of *O. europaea* subsp. *cuspidata* (Indian olive) seed extracts [13]. *In vitro* experiments showed 82.10% inhibition of α-amylase at 1.6 mg/mL. In *in vivo* experiments, rats were given a single intraperitoneal dose of alloxan monohydrate (150 mg/kg) to induce diabetes. Serum FBG levels were measured three days after the alloxan injection, and rats with FBG levels of 200 mg/dL were categorized as diabetic. Group 1 consisted of rats without diabetes receiving normal saline orally, while group 2 consisted of diabetic rats receiving glimepiride (0.2 mg/kg) orally as the usual reference.

Antifungal activity

Microbial infections have emerged as a significant global concern, with the *Candida albicans* species being one of the most prevalent fungal strains found on human mucosal membranes. This yeast can attach to epithelial tissues, resulting in superficial infections commonly referred to as candidiasis. Under certain conditions, candidiasis has become a major contributor to morbidity and mortality among immunocompromised individuals worldwide. A primary challenge lies in the multidrug-resistant nature of both bacterial and fungal strains. Consequently, researchers are increasingly turning to phytotherapy using medicinal plants, which possess antimicrobial properties due to their chemical compounds. In a similar way, additional researchers found that leaf essential oil has

promising antifungal properties [22]. However, a number of variables, including the kind of olive cultivar, the extraction method, the solvent, and the harvesting time of the byproduct, can impact how effective these extracts are. Olive extracts from four cultivars were tested against different fungal strains. Variations in total phenolic content caused variations in the minimum inhibitory concentration (MIC), demonstrating the influence of cultivars on the effectiveness of biological activity [23]. Analyses of the phytochemical makeup and antibacterial qualities of olive leaf extracts made with water-immiscible solvents were conducted by Bawadekji *et al.,* [24] who found that *Aspergillus niger* and *Candida albicans* growth were significantly inhibited.

Antimicrobial activity

Different extracts of *O. dioica* bark were employed [25] to check the antimicrobial properties using various microorganisms attained from the Institution of Microbial Technology (IMTECH) viz., *Streptomyces pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Xanthomonas campestris, Pseudomonas syringae, Agrobacterium tumefaciens* and *Klebsiella pneumoniae and* fungal strains like *Candida albicans, Trichophyton rubrum, Chrysosporium merdarium* and *Chrysosporium keratinophilum* Table. 4.

Table 4: Antimicrobial activity

Test organisms	Activity of extracts		
	Petroleum ether	Chloroform	Methanol
Fungal strains			
C. keratinophilum	-	-	-
C. albicans	-	-	-
C. merdarium	-	-	-
T. rubrum	-	-	-
Bacterial strains			
S. aureus	-	-	+
P. aeruginosa	-	-	+
S. pneumoniae	-	-	+
S. typhi	-	-	+
E. coli	-	-	+
X. compestris	-	-	+
P. syringae	-	-	+
A. tumefaciens	-	-	+
K. pneumoniae	-	-	+

Experiments were performed against test pathogens for all the three extracts of petroleum ether, chloroform and methanol using standard antibiotics; Fluconazole as a fungal drug and Ciprofloxacin as an antibacterial drug. Against all pathogens examined, the methanol crude extract exhibited significant antimicrobial activity but none of the extracts have shown the antifungal activity [11].

Anti-Alzheimer's activity

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Acetyl cholinesterase (AChE) breaks down acetylcholine, leading to cognitive decline. Inhibiting AChE can enhance cholinergic neurotransmission and improve symptoms.

Acetyl cholinesterase (AChE) inhibition assay

Pratap *et al.,* estimated acetyl-cholinesterase inhibitory activity with methanol extract of *O. dioica* leaves by bioautographic method and spectrophotometric method. The column chromatography fractions were subjected to TLC and examined for spots to assess AChE activity. The AChE inhibition was confirmed by exhibiting white spots or colorless regions over yellow background [15].

Inhibition of AChE using a bioautographic enzyme test

The bioautographic method [14, 26] states that TLC was used to find the acetylcholinesterase inhibitory activity of methanolic fractions. On a silica gel-coated TLC plate, 2.5 mm F-254, 10x10 cm (Merck, Germany), the methanolic fraction was spotted (10 g/mL) and developed using a standardizing mobile-phase ($CHCl_3$:MeOH, 9.5:0.5) before being allowed to dry at ambient temperature. The plates were then allowed to rest at room temperature for three minutes after being sprayed with a freshly prepared AChE (Electric eel AChE enzyme; Sigma-Aldrich) enzyme solution. After that, they were sprayed with 15 mM acetylcholine iodide (ATCI) solution in phosphate buffer (pH 7.2) and 3 mM DTNB or Ellman's reagent until they reached saturation. The plates were subsequently dried at room temperature for one minute to demonstrate the existence of suspected AChE inhibition zones, producing colorless or white specks on a yellow background. The spots on the plates were identified as clear zones against a yellow background and confirmed to be potential AChE inhibitors after the R_f value of the plant bioactive components that were computed for the molecule's separation in preparative scale was measured [14, 27, 28].

Determination of total flavonoid content

Various plants contain bioactive compounds with AChE inhibitory activity. Examples are alkaloids, flavonoids, terpenoids, and polyphenols. The potential candidates are: Galantamine (from snowdrop), Huperzine A (from *Huperzia serrata*). Natural inhibitors offer lower toxicity

and additional neuroprotective benefits. Calculating the total amount of flavonoids in *O. dioica* extract, as a reference, quercetin was used to calculate the total flavonoid content. In short, 300 µl of sodium nitrite (5%) and 300 µl of aluminium chloride (10%) were combined with 5 ml of extracts (200 µg) in Millipore water. The reaction mixture was then allowed to stand at room temperature for 6 minutes before 2 ml of sodium hydroxide (1 M) was added. Later, 2.4 ml of Millipore water was added to each test tube to bring the volume up to 10 ml. At 510 nm, absorbance was measured in relation to the blank. The total flavonoid concentration of the extract was measured in EQ, or equal to quercetin (µg/mg of dry mass) [16, 29].

In vitro cytotoxic activity

Trypan blue dye exclusion method [16] and 3-(4, 5 dimethylthiazole-2yl)2, 5-diphenyltetrazolium bromide assay [30] were used to assess *in vitro* cytotoxicity. These tests indicated that the ethanolic bark extract has a good cytotoxic action, and that its pure component, benzene-ethanol, 4-hydroxyalcohol, also has an outstanding activity that is equivalent to normal curcumin [10].

Aphrodisiac activity

Aphrodisiac is an activity wherein it penetrates the blood-brain barrier and imitates or activates some region of sexual arousal in the central nervous system, it increases sex drive or sexual pleasure [31]. Using several conventional aphrodisiac criteria, Ashwathanarayana and Naika have evaluated the effects of *O. dioica* Roxb., leaf and bark extract and its separated component benzene-ethanol, 4-hydroxy- on aphrodisiac activity in rats. On Wistar albino rats, the aphrodisiac activity of *O. dioica* Roxb., leaf and bark extracts, as well as its pure component, was investigated at various doses. When compared to ethanol bark extract and the pure component benzene ethanol 4-hydroxy, the results revealed that *O. dioica* Roxb., leaf and bark ethanolic extracts have good aphrodisiac effect [32].

CONCLUSION

In non-industrialized civilizations, medicinal plants are extensively utilized, mostly because they are easily available and less expensive than contemporary medications. Different parts of the plant of *O. dioica* such as leaves, bark, root, and fruits are treated as traditional medicine to cure fever, cancer, snake bite, rheumatism and skin diseases and many more. It is described to contain carbohydrates, flavonoids, fats, steroids, phenolic compounds, proteins, saponins, reducing sugars alkaloids and minerals. The therapeutic value of *O. dioica* has been demonstrated in pharmacological investigations and presented in this review. It is a significant source of a wide range of chemicals with a wide range of chemical structures and pharmacological activity. The presence of such a diverse spectrum of chemical compounds suggests that in future, the plant might contribute as a "lead" for the creation of new medicines with high efficacy in a variety of diseases.

DISCLOSURE

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None.

Conflict of Interest

The authors declare that there is no conflict of interest.

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