JAPR

***Vol. 03, Issue, 02, pp. 6-12, 3** June, 2026*

https://doi.org/10.66553/japr.2026.127

REVIEW ARTICLE

Exploring the Antimicrobial Potential of Olive Leaves; Alternative to Antibiotics

Sahiba Muhammad Khan[a] *, Muhammad Shafee[a], Shabir Ahmed[a], Muhammad Naeem[a], Munir Ahmad Khetran[b], Farooq Shahzad[c], Maham Sheikh[a]

a Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), Discipline of Microbiology, University Balochistan, Quetta, Pakistan.
b Balochistan Agricultural & Research Development center (BARDC), Brewery Road, Quetta, Pakistan.
c Deputy Director Agriculture Extension and Cooperative Department, Quetta, Pakistan.

ARTICLE INFO

Article History:
Received 5 *May*, 2026
Received in revised form 16 *May*, 2026
Accepted 30 *May*, 2026
Published online 3 *June*, 2026

Keywords:
Antimicrobial potential
Olive leaf extract
Phenolic compounds

Corresponding authors: Sahiba Muhammad Khan
Email**:** sahibabarech19@gmail.com

ABSTRACT

Olive leaves are rich source of phenolic compounds that have strong biological and antimicrobial effects. In the current global antimicrobial resistance threat, the search for alternatives to antibiotics is increasing. Olive leaf has been studied as an important cost-effective medicinal source of natural antimicrobials. This present review reports on current findings on the most isolated phenolic compounds from olive leaf extract with their antimicrobial potential and factors influencing the phenolic profile. This paper uses recent research studies with a wide range of experimental models to describe the antimicrobial potential of these phenolic compounds. It also describes the vast range of information about methods used to asses antimicrobial potential focusing on effects on different microbes. Furthermore, the review highlights the values of olive leaves as an agricultural and industrial byproduct, discussing their availability, therapeutic, nutritional benefits and research studies supporting their use.

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1. Introduction

The *Olea europaea* L. (olive) trees are native to tropical and temperate zone areas that belong to *Oleaceae* family. In which the most important and valuable member is *Olea europaea* [1]. Olive trees are cultivated approximately in over 60 countries in five continents with more than 2000 cultivars recognized globally [2]. Olive trees are distributed mainly in Greece, Spain and Italy. Olive leaves have long history of medicinal use across the old ancient civilizations. The ancient Egyptians used the for mummification of pharaohs. Later, it became a folk treatment for fever. The British people also used olive leaves to treat tropical diseases like malaria etc. By the mid-20th century, through research olive leaf extracts have shown to be used to manage hypertension. Since then, scientific interest in the therapeutic potential of olive leaves have been increased [3]. Recent years have highlighted the health benefits of regular consumption of medicinal herbs, fruits, and vegetables. These benefits largely come from their composition and high content non-nutrient bioactive compounds, such as phenolics. Phenolic compounds possess the abilities to modulate several biological processes and pathways in the body, including glucose levels regulation, boosting anti-inflammatory, antioxidant, anti-mutagenic, anticancer and neuroprotective properties [4]. Polyphenols, the secondary metabolites produced by plant are present in 43 different substructures that falls into five main classes: (i) flavonoids, (ii) lignans, (iii) phenolic acids, (iv) stilbenes, and (v) other PPs [5]. However, it can be primarily divided into two main types: flavonoids and non-flavonoids. Each type is further divided into different subclasses on the basis of phenol unit's structure, number of substituent groups attached and the types of linkage [6]. Polyphenols are widely found in different parts of plants such as peels, husks, seeds, and leaves. Plants produce an estimated 450,000 secondary compounds, with approximately 200,000 already identified and classified. These compounds serve many roles, such as defending both plants and animals against biotic and abiotic stresses [7].

Olea europaea L. is a woody oil producing tree native to the Mediterranean region and is best known for producing extra virgin olive oil. The leaf shape and size may vary based on tree's age, growing conditions and pruning methods. The olive fruit morphology is not distinct, but the chemical composition and organoleptic qualities are unique. Therefore, the nutritionists and the cardiologists consider olives for their potential health benefits [8].

Olive leaf extract is a bitter tasting dark brown liquid and is recognized in natural medicine and has been traditionally used as an herbal supplement. Due to the polyphenol richness, it has many beneficial properties such as boasting energy, regulating blood pressure, supporting both the cardiovascular and immune functions. Like any other natural products, the chemical composition of the extract may vary according to different olive cultivar type, geographical location and nutrition of plant [9]. The crushed olive leaf was used in drink form to lower the fever condition. olive tree leaves possess many benefits in cosmetic, food and nutraceutical applications. Olive leaf extract (OLE) is very effective against many diseases like coronary artery disease, hypertension, elevated cholesterol level, arrhythmia, osteoporosis, cancers, diabetes and overweight. The OLE has recently been given in the form capsule to make the administration easy. The antimicrobial and antioxidant activity of OLE are directly related with its phenolic content. In literature different studies have revealed that the olive polyphenols can stop the *Bacillus cereus* sporulation, suppress the growth of all known food pathogens [10]. The primary classes of phenols in OLE are secoiridoids, flavonoids, phenolic alcohols and phenolic acids [11-12]. Due to increased phenolic content and antimicrobial and antioxidant potential the OLE is used as food additive. It contains naturally occurring phenolic compounds and recognized for its beneficial physiological and biological qualities, such as antimicrobial, anti-inflammatory, antiarthritic, antiallergenic, anticarcinogenic and antihypertensive activities. The most abundant biophenol is oleuropein, a secoiridoid, composed of hydroxytyrosol and elenolic acid, that is responsible to make olive fruits taste bitter. It has high concentration particularly in green olives it is up to 1–2 percent while different other phenols are present in low quantity such as apigenin-7-glucoside, verbascoside, hydroxytyrosol and luteolin-7-glucoside [13]. Oleuropein can be cleaved to elenolic acid, hydroxytyrosol, oleuropein aglycone and glucose. It is a complex but major phytochemical and found in high amounts in olive leaf but low amounts in olive oil [14]. The olive leaves are broadly used as a vasodilatory, antirheumatic, diuretic, antipyretic agent. Broad spectrum of pathogens like bacteria, viruses and even various parasites are sensitive to oleuropein from OLE but the mechanism of action is not fully explained. Some researchers have attributed it to the ortho-diphenolic system (catecin) [15].

Olive leaves are orally taken for intestinal diseases, stomach, bronchial asthma and chewed as a mouth cleanser. The *olea europaea* leaf decoctions are taken orally for urinary tract infections and diarrhea [16]. As described in different research studies oleuropein is shown to have anticlastogenic activities, inhibits the development of various tumors, inhibit oxidation of low-density lipoprotein and lipoxygenases. Due to the ability of improving lipid metabolism, it facilitates in obesity [3]. OLE has been reported to have antiviral activity, because of calcium elenolate and elenolic acid derivative and may have activity against all viruses tested, such as Herpes simplex type I, II, Herpes zoster, myxoviruses, Polio 1, 2, and 3 viruses, rhinovirus, two strains of leukemia virus, different strains of influenza and para influenza viruses and Encephalomyocarditis [17].

Table 1. Biological activities and potential health benefits relating to olives phenolics [18]

Biological Activity	Potential Clinical Target
Antioxidant activity	Cardiovascular and degenerative diseases
Anti-inflammatory activity	Inhibition of pro-inflammatory enzymes
Antimicrobial activity	Infectious diseases
Anti-atherogenic activity	Coronary heart diseases, stroke
Anti tumor activity	Various cancers
Anti platelet aggregation	Coronary heart diseases, stroke
Anti-hypertensive activity	Hypertension
Increased vitamin A and β-carotene activity	Antiaging/skin protection
Increased immune activity	Infectious diseases; various cancers
Anti-allergic activity	
Reduction in the levels of plasma cholesterol and oxidized LDL	Coronary heart diseases

2. Main Phytochemicals of Olive Leaves and Their Biological Effects

Olive leaves contain phenols similar to those in the olive oil or fruit but in a much higher concentration. As a result, olive leaf extract may possess more potential to offer great benefits for health outcomes than virgin oil [Table-1]. The most abundant phytochemical in olive leaf is oleuropein. Due to the high phenolic profile of olive leaves, it provides several health benefits such as antioxidant and antimicrobial potential [1]. The polyphenols in olive leaves have been extensively studied for its dietary purposes, as olive fruits and oils are staple food for a huge part of the global population. In contrast, olive leaves are valued for their secondary metabolites, particularly the oleuropein, secoiridoid and oleacein. Traditionally, olive leaves have been used as an herbal remedy and are known for their

positive effects on metabolism [19]. Olive leaf biophenols are believed to be responsible for therapeutic effects. Oleuropein the primary constituent exhibits cardioprotective, antiviral, antimicrobial, antihypertensive, antioxidative and antiatherogenic activities. Secoiridoids is a group of coumarin-like substance, and which is abundant not only in olive but also in many other plants from the Cornales, Oleaceas, Gentianales and other plant species. Secoiridoids and iridoids typically occur in glycosidic forms. They originate from the secondary metabolism of terpenes and serve as a precursor for various indole alkaloids [3]. Oleuropein is an iridoid monoterpenes phenol found is olive leaves and fruits that is formed from hydroxytyrosol esterified with α-glucosylated elenolic acid. It is considered the main compound responsible for the pharmacological properties of the olives. Oleuropein have strong anti-inflammatory and anti-oxidant properties that can help in the treatment of cardiovascular disease, obesity, diabetes and hepatic disorder [20-21].

Olive leaves are rich in flavonoids, which make up the most abundant and widespread group of polyphenols in olive leaves [22]. Recent studies in both human and animals showed that flavonoids are functional bioactive compounds capable of strengthening antioxidant defenses, reduce the risk of mutations, free radical damage, chronic inflammation. Due to its potential health-promoting effects, nutritional benefits, pharmacokinetic behaviors and therapeutic mechanisms, flavonoids are among the most extensively researched natural compounds [4].

Luteolin is another significant flavonoid commonly found in many plants including, broccoli, carrots, cabbages, chamomile tea, apple skins, celery, olives and onion [23]. Plants rich in luteolin have long been used in traditional medicine to treat different inflammatory conditions, research suggest that, luteolin potentially controls colon cancer in multiple aspects [24]. Active phenols and flavonoids compounds are capable to damage the cell walls of bacteria, Alkaloids and tannins inhibits key metabolic enzymes in bacterial cell, while steroids and fatty acids increase membranes permeability of bacterial cell, thus impairing cell functions. Together, these mechanisms highlight the importance of secondary metabolites as broad spectrum natural antibacterial agents [25]. Recent research reported that olive leaf has a more polyphenols than olive oil and the whole fruit [26].

Table 2. Characterization of phenolic compounds from olive leaves via HPLC–ESI–MS. [27]

No	Proposed Compounds	Rt min	*m/z* Experimental	Class
1	Loganic acid	4.28	375.1	Iridoids
2	Hydroxytyrosol	5.543	153.1	Simple phenols
3	Esculin	6.14	339.2	Coumarins
4	Taxifolin-3-glucoside	6.451	465.2	Flavonoids
5	Chlorogenic acid	7.003	353.2	Hydroxycinnamic acid
6	Secoxyloganin	8.359	403.2	Iridoids
7	Luteolin-3′,7-di-O-glucoside	9.069	609.1	Flavonoids
8	Plantamajoside	9.55	639.3	Hydroxycinnamic acid
9	Rutin	11.62	609.3	Flavonoids
10	Luteolin rutinoside	11.767	593.3	Flavonoids
11	Quercetin-3-O-glucoside	12.342	463.2	Flavonoids
12	Luteolin-7-O-glucoside	12.613	447.4	Flavonoids
13	Apigenin-7-O-neohesperidoside	13.546	577.3	Flavonoids
14	Taxifolin	13.793	303.3	Flavonoids
15	Quercetin 3-O-rhamnoside	14.347	447.2	Flavonoids
16	Apigenin-7-O-glucoside	14.47	431.4	Flavonoids
17	Kaempferol-7-O-glucoside	14.697	447.2	Flavonoids
18	Luteolin-4’-O-glucoside	15.733	447.3	Flavonoids
19	Oleuropein isomer 1	16.419	539.2	Iridoids
20	Oleuropein isomer 2	17.089	539.2	Iridoids
21	Oleuropein isomer 3	17.647	539.2	Iridoids
22	Eriodictyol	20.765	287.1	Flavonoids
23	Luteolin	21.133	285.5	Flavonoids
24	Quercetin	21.35	301.3	Flavonoids

25	Kaempferol	24.85	285.4	Flavonoids
26	Hispidulin	27.701	299.3	Flavonoids
27	Asiatic acid	38.11	487.3	Triterpenic acids
28	Oleanonic acid	38.71	455.3	Triterpenic acids
29	Maslinic acid	39.64	473.5	Triterpenic acids
30	Corosolic acid	40.19	473.2	Triterpenic acids

3. Effect of Extraction method and solvent type

Different extraction parameters are influencing the phytochemical content of olive leaves, such as solvent type (ethanol, acetonitrile, distilled water), extracts preparation method (sonication, maceration, two steps maceration), pH and temperature. By using HPLC, the quantitative and qualitative analyses of phenolic compounds were performed. Research findings indicated that the dried leaves extract obtained by two steps maceration in ethanol followed by distilled water showed increased flavonoids and phenol content as compared to those extracts obtained by the other solvents and techniques. The macerated extract was further investigated for its antibacterial potential against five pathogens (*L. monocytogenes, E. coli, P. aeruginosa, S. aureus* and *B. subtilis*) which showed strong antibacterial effectiveness due to the increased concentration of oleuropein and other active compounds [28]. Another researcher reviewed fourteen studies with 149 olive leaf samples included to see how extraction method, solvent type, and extraction conditions such as time and temperature effects the recovery of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity from olive leaf extracts. Result showed that with optimized parameters, both conventional and assisted extraction methods can yield high phenolic content, though the outcomes can vary due to differences in methodologies [29].

4. Factor effecting phenol content in olive leaf extract

Genotypeic information of olive trees are critical in profiling the phenolic compounds and their antioxidant activity[30]. Several other factors can alter the synthesis and content of phenols in olive, including geographical zone, salinity, hydric deficiency, fertilization, frost stress, light exposition, bacteria, fungi, tree genotypes, leaves age, alternate bearing patterns and sampling time [31]. All olive oils have different phenolic profile, hence different bactericidal effects. Olive cultivar affects the composition of olive oil and not every olive cultivar possess antimicrobial activity [32]. Temperature has strong effect on extraction outcomes. Extractions above 50 °C yields five times higher total phenolic compounds than at room temperature [29].

5. Antimicrobial activity

OLE possess activity against microorganisms that are commonly linked with respiratory and gastrointestinal tract infections. The OLE antimicrobial potential is credited to its phenolic constituent synergistic combination such as hydroxytyrosol and oleuropein and some other components like fatty acids. According to latest studies, antimicrobial activity of OLE can inhibit biofilm formation up to 82 % at sub-inhibitory concentrations in multi-drugs resistance (MDR) strains of *P. aeruginosa* [33]. The polyphenols in olive leaves possess activity against different microbes including gram negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*), gram positive bacteria (*B. cereus, B. subtilis* and *S. aureus*) and fungi (*Candida albicans* and *Cryptococcus neoformans*) [34]. Similarly, OLE has antimicrobial activities against foodborne pathogenic bacteria such as *L. monocytogenes*, *E. coli, S. aureus* and *salmonella* spp [35]. Oleuropein and other phenols have demonstrated antimicrobial potential against bacteria, fungi, viruses and parasites. Phenols in olive fruit and foliage have inhibition effects against *K. pneumoniae, B. cereus*, *E. coli* and *S. aureus* [36,39]]. Pasandideh & Anvari, (2018) reported that olive-based products, such as olive leaf extracts and oils exhibit strong antimicrobial potential due to its high oleuropein content [37]. Another study also reported that the oleuropein possesses strong antioxidant and antimicrobial activities [3]. Olive leaf extracts and extra virgin olive oils possess effective polyphenols their mechanisms of action found were quorum sensing inhibition, virulence factors inhibition, biofilm formation inhibition, damage to cell wall, increased membrane permeability [40]. The antimicrobial effectiveness of OLE combined with nanoparticles can further be enhanced against resistant strains [38-39].

6. Olive cultivation in Pakistan

Olea europaea L. cultivation has remarkable capability for diversifying agriculture and boosting economic growth in Pakistan, especially in semi-arid and rainfed regions. In Punjab, the Potohar region has become a potential zone for olive cultivation due to its favorable climate. In Chakwal the Barani Agriculture Research Institute (BARI), has played a significant role in the development and evaluation of olive cultivars suited to Pakistan, such as BARI Zaitoon-1 and BARI Zaitoon-2. In Potohar research trails, cultivars like Earlik, Hamdi, Ascolana and Gemlik have performed well and shows potential for commercial orchards due to their good oil recovery and strong fruit yield. In Pakistan, olive cultivation can contribute to country's cooking oil supply, offer farmers a durable income-generating crop and generate employment in rural areas. In last few years, the government of Pakistan has initiated a billion olive tree drive with everlasting effects.

6.1 Olive Cultivation in Balochistan

In Balochistan, olive farming is getting traction due to the province's dry climate, highland terrain and availability of land suitable for orchards. Important and potential area for olive productions is the Loralai including, Zhob, Khuzdar, Killa Saifullah and Musa Khel. Among these Loralai is the major production Centre. In Quetta, at the Balochistan Agriculture Research and Development Centre (BARDC), research has conducted recently to evaluate olive cultivars such as Arbosana, Arbequina, Leccino and Pandolino. Results showed that Leccino and Pandalino adapted best to Quetta and surroundings agro-ecological conditions. Similarly, Pandalino gave the best highest fruit weigh per plant among the cultivars tested, while Leccino had an oil content of around 15%. Broadening olive cultivation could help diversify Balochistan's agriculture, raise farmers income, support local edible oil production and utilize the dry lands productively.

7. Conclusion and recommendation for Future work

Olea europaea L. leaves are an important and valuable natural source of bioactive compounds that exhibit strong antimicrobial activities. They are rich in phenolic substances such as oleuropein, hydroxytyrosol, flavonoids and antioxidants that possess the potential to inhibit the growth of various pathogenic bacteria and fungi. These antimicrobial effects are likely due to the olive leaf's phytochemicals that are capable of damaging the microbial cell membrane, disturbing key metabolic functions and preventing microbial growth. Though olive leaves are usually considered as an agricultural by-product, they have great potential to be used as natural food preservatives, medicines and other health products. Additional research is required to evaluate the use of olive oil extracted left over and leaves to be used as feed for animals and other purposes that will play a significant role in the uplift of socio-economic standard of poor farmers of the region.

Authers contribution

Sahiba Muhammad Khan: Original draft writeup, **Farooq Shahzad:** Reviewing & Editing, **Muhammad Shafee, Munir Ahmad:** Conceptualization & Reviewing, **Shabir Ahmed:** Investigation and reviewing, **Muhammad Naeem:** Reviewing & Editing, **Maham Sheikh:** Data collection.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Declaration of conflict of interest

The authors no conflict of interest.

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