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Utilizing the potential of *Saussurea lappa* in aquaculture industry: a review on immune enhancement and pollution remediation

Mohammed Abd-Elhady Naiel[1] · Abdelrazeq Mohamed Shehata[2,3] · Vinod Kumar Paswan[3] · Mourad Ben Said[4,5] · Khaled A. El-Tarabily[6,7]

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Abstract
Aquaculture and the production of aquatic goods are rapidly growing industries in the world. These industries involve the cultivation of various saltwater and freshwater fish as well as shellfish species, and they undergo regular updates in their manufacturing processes. The increasing popularity of aquaculture is driven by the desire to achieve long-term sustainability in food production. However, the intensification of farming techniques can lead to economic losses due to fish mortality, which is often caused by infectious and stressful conditions. To address this challenge, it is crucial to enhance the immune response of fish as part of sustainable intensification and aquaculture management. Recently, there has been growing interest in eco-friendly and natural bioactive solutions as potential alternatives to synthetic compounds used for disease prevention in aquaculture. Medicinal plants, available as whole plants, plant extracts, or synthetic analogues of natural substances known as phytomedicines or phytopharmaceuticals, have shown high efficacy in disease prevention for humans and animals. One such medicinal plant is *Saussurea lappa*, which is extensively utilized in various forms of medicine for treating multiple diseases. The bioactive chemicals derived from *S. lappa* exhibit a wide range of biological activities, including anti-inflammatory, antioxidant, antimicrobial, and anticancer properties, along with immunostimulatory effects. Given the diverse biological activities of *S. lappa* and its potential to enhance the immune responses of aquatic species, this review focuses on exploring its contributions in this area. By examining the numerous benefits and applications of *S. lappa*, we aim to shed light on its potential role in improving the immune response of aquatic species.

Keywords Aquaculture industry · Bioactive solutions · Disease prevention · Immune response · *Saussurea lappa*; sustainable intensification

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Extended author information available on the last page of the article

Introduction

Economic losses incurred in aquaculture due to fish mortality are a drawback associated with intensive farming. These farming systems have the potential to elevate the likelihood of infectious diseases and stressful conditions for the fish (Guardiola et al. 2016). The well-being of aquatic organisms can be negatively affected by both physical and physiological challenges arising from disruptions in their aquatic environment. These disturbances can arise from factors such as inadequate treatment, overcrowding, transportation, infectious diseases, or deterioration of water quality (Eissa et al. 2014). Commercial aquaculture farms that involve rearing fish at very high densities require enhancement of the immune system of fish to maintain their healthy growth and allow them to overcome the different environmental stressors (Davis 2006), such as water-quality deterioration, and infectious diseases (Kelly et al. 2006).

According to the general definition of stress in fish, any external influences in the surrounding environment are known as stressors, and they impair the homeostasis of fish and cause behavioural, physiological, and neuroendocrine changes (Eissa and Wang 2016). The immune system is a vital component of the fish system within the aquaculture ecosystem, serving a crucial role in responding to diverse stressors (Wecksler 2010). The impairment of immune systems in aquatic animals carries significant economic consequences as it destabilizes aquatic systems, adversely affecting fish health, production, and quality (Mohan and Bhatta 2002).

Moreover, adverse effects of environmental stressors and infectious diseases are believed to be manifested by multiple mechanisms. The total effects resulting from environmental stressors are measured by their capability to impair biological systems either functionally or structurally or in both ways (Fatima et al. 2000). Furthermore, considering that antibiotic resistance is an emerging concern, which presents a universal threat to both humans and animals by hindering the control of bacterial diseases, alternative strategies for pathogen control are needed (Arias and Murray 2012).

Natural compounds derived from medicinal plants exhibit antimicrobial properties against various microorganisms that affect humans, plants, and fish, including antibiotic-resistant strains like *Enterococcus faecalis*. These microbial strains demonstrate a high susceptibility to organic extracts (using methanol or acetone) as well as aqueous extracts from numerous medicinal plants (Rahman et al. 2017).

Nowadays, there is a growing demand for fish products that are free of pollutants and antibiotic residues, which has led to an increase in the use of therapeutic herbs from medicinal plants in aquatic projects. Such natural compounds are adequate substitutes for chemical and hazardous additives (Adel et al. 2016). Many studies have reported that medicinal plants are effective for disease prevention and control because of their antioxidant, germicidal and immunomodulatory properties (Citarasu 2010; Chakraborty and Hancz 2011; Adel et al. 2016). In the field of aquaculture, boosting the immune system of fish by supplementing their diet with natural compounds is viewed as a promising strategy for mitigating environmental stressors and managing various diseases (Sakai 1999).

These natural compounds have gained popularity as alternatives to traditional chemotherapeutic agents and vaccines (Secombes 1994). The functioning and effectiveness of the immune system can be influenced by a wide range of both internal and external factors possessing either immunosuppressive or immunostimulant properties (Chakraborty and Hancz 2011). Numerous natural bioactive compounds, called immunomodulatory agents, can prevent pathogenic microorganisms and cancer-associated diseases, and thus, they can

modulate the immune system (Shukla et al. 2014). Furthermore, natural bioactive compounds may decrease treatment costs and are safer for the environment because of their higher biodegradability than synthetic compounds (Zanuzzo et al. 2015).

The diverse array of plant extracts possesses a lower tendency to induce drug resistance in pathogens, distinguishing them from other pharmaceutical options (Reverter et al. 2014). Numerous medicinal plant extracts have been identified for their capacity to enhance immune responses and provide protection against various diseases when administered as supplements to cultured fish (Kiron 2012; Militz et al. 2014; Reverter et al. 2014). Indian and Chinese medicinal plants, in particular, have been found to exhibit immunomodulatory activities (Jian and Wu 2004). Among these medicinal plants, *Saussurea lappa* exhibits various valuable biological actions and shows the potential to be applied as a substitute for chemotherapeutic agents in aquaculture. *S. lappa* is well known as a medicinal plant in different branches of medicine and can treat many diseases (Fan et al. 2014). Various bioactive molecules have been extracted from *S. lappa*, including costunolide, cynaropicrin, and dehydrocostus lactone (Fan et al. 2014).

Throughout history, medicinal plants have played a significant role in pharmacological therapies, and they are currently being recognized for their potential in providing synergistic combinations. This review article explores recent advancements in natural immunological and pharmaceutical research related to *S. lappa*, highlighting its contributions in these areas.

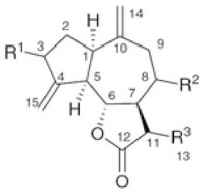
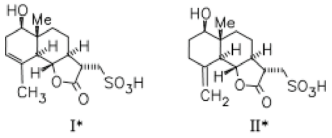
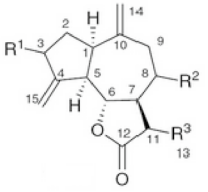
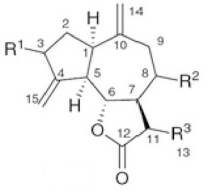
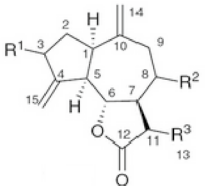
Chemical constituents of *S. lappa*

The genus, *Saussurea* DC., belongs to family Asteraceae, which contains around 300 species. *S. lappa* Clarke, commonly known as Costus, is one of the most common species within this genus (Xu et al. 2019). *S. lappa* produces an amazing variety of bioactive phytoconstituents that have gained importance due to their therapeutic and pharmacological properties. The most important bioactive compounds in this elite medicinal species include alkaloids, sesquiterpenes, flavonoids, acacetin, steroids, phytosterols, cynaropicrin, glycosides, lignans, polyene alcohols, mokko lactone, tannins, germacrenes, and chlorogenic acid (Lee et al. 1995; Taniguchi et al. 1995; Hui et al. 1997; de Kraker et al. 2001; Wang et al. 2010; Bains et al. 2019). Table 1 summarizes the list of the primary bioactive molecules identified from *S. lappa*.

These various components provide a wide range of biological activities to *S. lappa* medicinal products (Table 2) such as immunomodulatory, anti-carcinogenic, anti-inflammatory, antioxidant, anti-bacterial and other therapeutic properties (Negi et al. 2014). The main phytoconstituents of this species are sesquiterpene lactones, such as costunolide and dehydrocostus lactone, which have many pharmacological properties (Park et al. 2009). These sesquiterpenes roughly belong to three groups: guaiane, eudesmane, and germacrane, which are biosynthesised successively (Madkour 2020). Because of the instability of germacrane, half of the sesquiterpenes comprise guaianes and 40% are eudesmanes, while the remaining small proportion is composed of germacrene (Madkour 2020).

The antioxidant activity and chemical composition of essential oils extracted from the roots of *S. lappa* were analyzed at four different time points. The primary bioactive component identified in the extracts was dehydrocostus lactone, with the highest concentration observed in 3-year-old plants collected in April and December (Benedetto et al. 2019).

Table 1 List of the main bioactive compounds isolated from *Saussurea lappa*

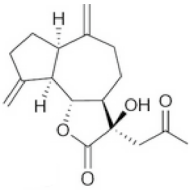
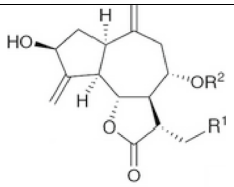
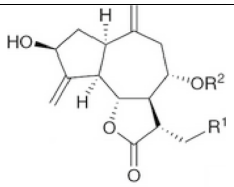
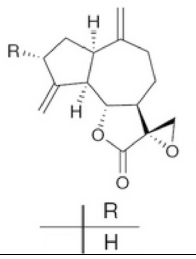
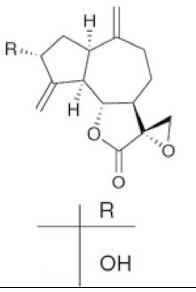
Compound	Structure	References
12-Methoxydihydrodehydrocostuslactone		Dhillon et al. (1987)
13-Sulfodihydrodehydrocostuslactone		Yin et al. (2005)
Sulfocostunolide-A		Yin et al. (2007)
Sulfocostunolide-B		Wang et al. (2008)
11β,13-Dihydro-3-epizaluzanin-C		Sun et al. (2003)

Mechanism of action

Toll-like receptors (TLRs) are the main mediators of innate immunity in vertebrates and invertebrates. Their activation is an initial step toward immune response and plays a central role in innate and adaptive immunity by enhancing antimicrobial activity and activating antigen-producing cells in fish (Zhang et al. 2014a). The response of TLRs to various pathogen-associated stimuli led to inflammation and the development of adaptive immunity. Nuclear factor-κB (NF-κB) is the main transcription factor that works on TLR signalling to control or trigger inflammation (Krishnan et al. 2007). Sesquiterpene lactones isolated from *S. lappa* may influence the immune responses enhanced by TLR-based signalling pathways (Krishnan et al. 2007).

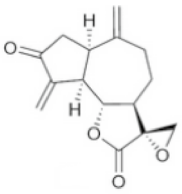
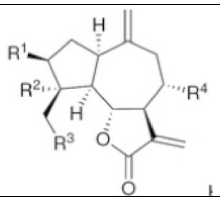
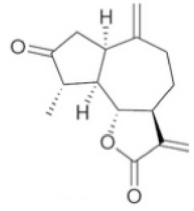
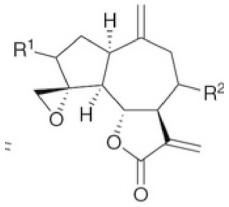
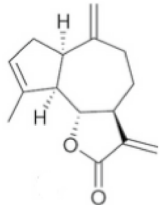
Sesquiterpene lactone extracted from *S. lappa* acts as a pharmacological suppressor of tumour necrosis factor–α (TNF-α) and NF-κB activation (Lee et al. 1999; Jin

Table 1 (continued)

Lappalone		Zhu et al. (2006)
Saussureamine B		Matsuda et al. (2000)
Saussureamine-C		Yoshikawa et al. (1993)
11,13-Epoxyisozaluzanin C		Chhabra et al. (1998a)
Epoxyisozaluzanin C 4α,15-epoxide		Robinson et al. (2010)

et al. 2000). TNF-α plays a dual role in inflammation injury, and the function of TNF-α depends on its concentration (Zhao et al. 2020). Inhibition of TNF-α or its receptor can control the content of lipopolysaccharides (LPS), inducing liver damage (Zhao et al. 2020). Various sesquiterpene lactones contain molecules that can form covalent bonds with functional protein molecules (Jin et al. 2000). NF-κB is a major regulator of the immune response and the inflammatory process (Liu et al. 2017). In addition, since NF-κB activation is frequently observed in inflammatory conditions associated with cancer, it represents a favourable therapeutic target with minimal risk of side effects (Taniguchi and Karin 2018). Consequently, the inhibition of NF-κB signalling pathway

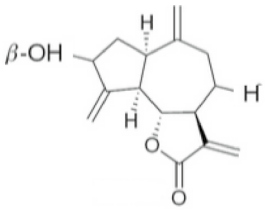
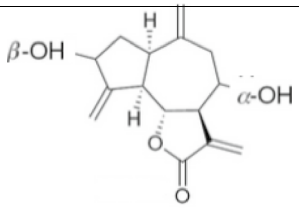
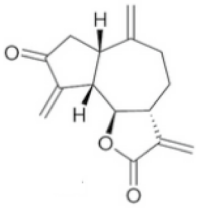
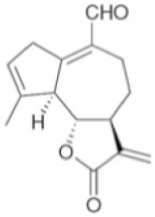
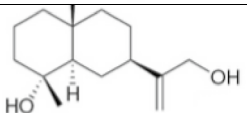
Table 1 (continued)

11,13-Epoxy-3-ketodehydrocostuslactone		Chhabra et al. (1998a)
15-Hydroxydehydrocostuslactone		Saxena and Dixit (1993)
4β,15-Dihydro-3-oxo-trans-germacran-6α,12-olide		Robinson et al. (2010)
Epoxyisozaluzanin C 11α,13-epoxide; 14,15-Epoxyisozaluzanin		Chhabra et al. (1998b)
Isodehydrocostuslactone		Kalsi et al. (1995)

could potentially serve as an effective strategy for anti-inflammatory therapeutic interventions (Taniguchi and Karin 2018).

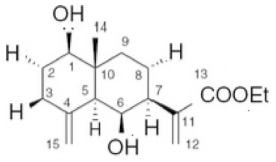
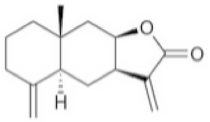
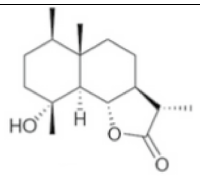
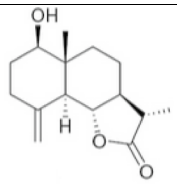
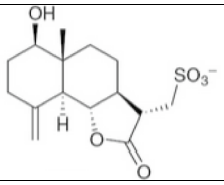
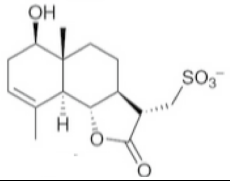
The expression of several pro-inflammation genes is related to nuclear translocation and DNA binding of NF-κB in the nucleus (Younus 2018). Suppression of DNA binding and nuclear translocation of NF-κB leads to the deletion or downregulation of NF-κB. The process of IkBα phosphorylation, an NF-κB inhibitor, by IKKβ results in the degradation of IkBα, inducing DNA binding and translocation of NF-κB in the nucleus (Giridharan and Srinivasan 2018). Dehydrocostus lactone has been reported to suppress LPS-induced IkBα degradation, leading to the suppression of DNA binding and nuclear translocation of NF-κB (Kim et al. 2020).

Table 1 (continued)

3-Epizaluzanin C		Kalsi et al. (1995)
Isozaluzanin C		Sun et al. (2003)
3-Oxodehydrocostuslactone		Chhabra et al. (1998a)
Saussurealdehyde		Kalsi et al. (1995)
4α-Hydroxy-4β-methyldihydrocostol		Duan et al. (2010)

Sesquiterpene lactones interact with the cysteine residue of the p65 subunit of NF-κB, leading to the inhibition of DNA binding, and thus, NF-κB action as well (Jin et al. 2000; García-Piñeres et al. 2001). The MyD88-dependent pathway of the action of TLRs stimulates NF-κB (Kawai and Akira 2007). Dehydrocostus lactone, extracted from *S. lappa*, suppresses the MyD88-dependent signalling pathway of TLRs by acting on IKKβ and interacting with two cysteine residues (Cys38 and Cys120) of p65/NF-κB, leading to the suppression of NF-κB (Kim et al. 2020).

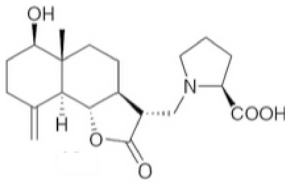
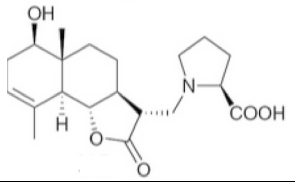
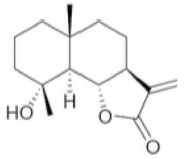
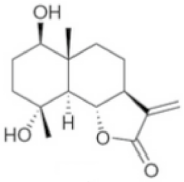
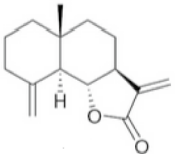
Table 1 (continued)

1β,6α-Dihydroxycostic acid ethyl ester		Sun et al. (2003)
α-Cyclocostunolide		Julianti et al. (2011)
Colartin		Sun et al. (2003)
11β,13-Dihydroreynosin		Sun et al. (2003)
13-Sulfodihydroreynosin		Yin et al. (2005)
13-Sulfodihydrosantamarine		Yin et al. (2005)

Administration and application techniques

Applying synthetic compounds in fish farm practices has well-known desirable impacts under suitable environmental conditions (Shohreh et al. 2023). Still, their recommendation for wide application in fish production is being confronted with limitations owing to their public health hazards (Idowu and Sogbesan 2017). There are numerous threats to both aquatic animals and the ecosystem, including the emergence and development of antimicrobial-resistant pathogenic microorganisms and mutant genes, as well as the accumulation of chemical compound residues in fish flesh and the ecosystem (Naiel et al. 2023a).

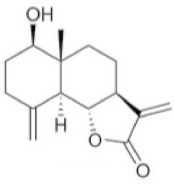
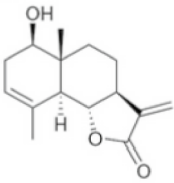
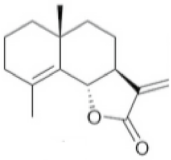
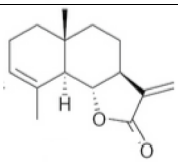
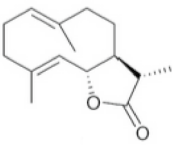
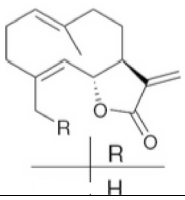
Table 1 (continued)

Saussureamine E		Matsuda et al. (2000)
Saussureamine D		Yoshikawa et al. (1993)
Arbusculin A		Sun et al. (2003)
1β-Hydroxy arbusculin A		Choi et al. (2008)
β-Costic acid		Govindan and Bhattacharyya (1977)

However, there has been minimal research into the dangers of using synthetic substances on farmed fish. There are indications that some could cause numerous negative consequences such as decreasing larval growth, nephrotoxicity, and disabling immunological protective mechanisms (Soliman et al. 2020; Subaramaniyam et al. 2023). Furthermore, the indiscriminate use of synthetic substances such as antibiotics in preventative therapy has resulted in the development of resistant bacterial strains and the need to switch to alternative medicines (Das Sarkar et al. 2020). Many of the synthetic chemicals also generate sensitization reaction and other undesirable side effects (Citarasu 2010).

As a result, it is critical to eliminate the widespread use of synthetic molecules in aquatic practices and direct efforts toward the discovery of new natural, less expensive, safer, and more environmentally friendly chemicals for the development and sustainability of the aquaculture business (Naiel et al. 2023b). Biomolecules or -by-products that are derived from natural resources, have been recognized as a suitable alternative source for synthetic chemicals application in farmed fish (Naiel et al. 2021; Sheikhzadeh et al. 2022).

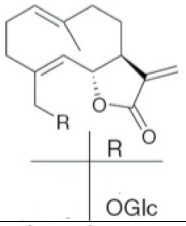
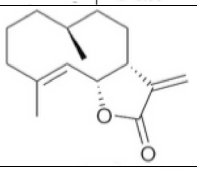
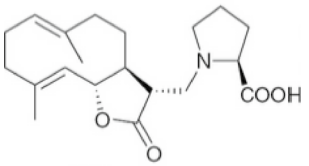
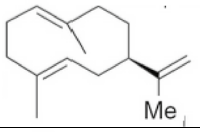
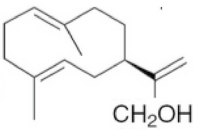
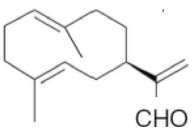
Table 1 (continued)

Reynosin		Choi et al. (2008)
Santamarin		Duan et al. (2010)
Arbusculin B		Julianti et al. (2011)
β-Cyclocostunolide		Duan et al. (2010)
11β,13-Dihydrocostunolide		Saxena and Dixit (1993)
Dehydrocostuslactone		De Kraker et al. (2001)

Medicinal plants and their by-products are specified specifically because they are easily obtained, inexpensive, profitable for a wide range of applications, and have an eco-friendly value because they are biodegradable (Kesbiç et al. 2022; Tarricone et al. 2023).

On the other hand, it was previously approved that a variety of biotic and abiotic environmental conditions such as, soil fertility and cultivation region influence the quantity, quality, and structure of secondary metabolites in medicinal plants (Vishvamitera et al. 2023). Because plants primarily use nitrogen (N), potassium (K), and phosphorus (P) to produce enzymes and amino acids necessary in the manufacture of the many important oil components, soil fertilization can successfully enhance the quality and productivity of

Table 1 (continued)

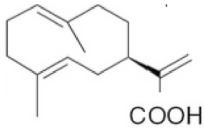
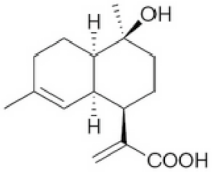
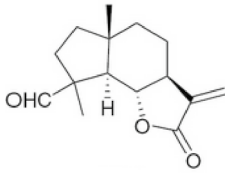
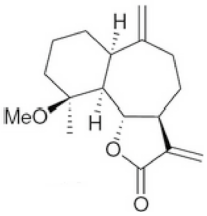
Picriside B		Yoshikawa et al. (1993)
Isodihydrocostunolide		Robinson et al. (2008)
Saussureamine A		Yoshikawa et al. (1993)
(+)-Germacrene A		De Kraker et al. (2001)
Germacra-1(10),4,11(13)-trien-12-ol		De Kraker et al. (2001)
Germacra-1(10),4,11(13)-trien-12-al		De Kraker et al. (2001)

medicinal plants (Soltanbeigi 2020). Strikingly, *S. lappa* grown in the Southwest China regions possesses potent antibacterial, antihypertensive, and spasmolytic characteristics (Zhao et al. 2017).

Furthermore, *Saussurea pulchella*, which is grown in Korea, has shown to have a variety of biological properties, including the ability to diminish inflammation, lower blood pressure, treat hepatitis, and prevent arthritis (Zahara et al. 2014). The Tibetan-grown *Saussurea laniceps* is also used to treat gynopathy and rheumatoid arthritis in the Chinese provinces of Yunnan and Sichuan (Nurzynska-Wierdak 2013). Meanwhile, *Saussurea involucrate*, a traditional Chinese medication from the Xinjiang Uygur Autonomous Region, has been used to alleviate stomach pain and rheumatoid arthritis (Verma and Shukla 2015).

Similar to other bioactive substances, the various bioactive compounds derived from *S. lappa* can be administered through different methods, including injections (Ponce et al. 2020), incorporation as additives in fish feed (Reverter et al. 2014), or immersion (Militz et al. 2014). Intraperitoneal injections have been demonstrated to be the most effective and

Table 1 (continued)

Germacra-1(10),4,11(13)-trien-12-oic acid		De Kraker et al. (2001)
10α-Hydroxyartemisinic acid		Duan et al. (2010)
Saussureal		Talwar et al. (1992)
4β-Methoxydehydrocostuslactone		Singh et al. (1992)

rapid mode of administration. However, this approach requires additional labor and costs, and it can induce stressful conditions for fish, particularly for fry and fingerlings. Consequently, the administration of *S. lappa* extracts in aquaculture systems as feed additives is more commonly used and considered suitable (Van Hai 2015). Extensive research was conducted on numerous medicinal plants to isolate several antibiotics, such as ampicillin, terramycin, and tetracycline. (Tadese et al. 2022). In light of the fact that there is currently no evidence to suggest that *Saussurea* species contain any kind of antibiotic, it is advised that they be utilized in aquaculture methods over an extended period of time (Tadese et al. 2022).

Furthermore, bioactive compounds derived from *S. lappa* can be used alone or in combination with other substances. Both approaches are similarly functional and reasonable. However, the administration of the different extracts of *S. lappa*, as well as a combination of its bioactive compounds, can exert a synergistic effect and provide several benefits to the aquatic fish such as, Tilapia (Ardó et al. 2008) due to the diversity of their different mechanisms of action (Pandey et al. 2007). In addition, extracts of *S. lappa* can be mixed with extracts of other medicinal plants or micronutrients and then administered to the host in order to provide a variety of benefits (Van Hai 2015).

The utilization of medicinal plant extracts for immune stimulation or the treatment of fish diseases using current methods is often considered non-palatable, ineffective, and costly. Moreover, there is a potential risk to the environment associated with their

Table 2 *Saussurea lappa:* alleged therapeutic claims and pharmacological evidence

Bioactive compounds	Administration	Experimental design	Results	References
Santamarin isolated from *S. lappa*	RAW264.7 cells were incubated with numerous concentrations of santamarin (5–80 µM) for 24 h	Lipopolysaccharide -stimulated RAW264.7 cells and murine peritoneal macrophages	Inhibited inducible nitric oxide synthase, decreased inducible nitric oxide synthase-derived nitric oxide, inhibited COX-2 protein and reduced COX-derived PGE2 production Reduced the production of TNF-α and IL-1β	Choi et al. (2012)
Sesquiterpene lactones compound (Dehydrocostus lactone) isolated from *S. lappa*	Dehydrocostus lactone (10 µM)	Murine macrophage cell line (RAQ 264.7) stimulated with Lipopolysaccharide 1 µg/ml Male ICR mice (25–30 g) injected with Lipopolysaccharide (2 mg/kg)	Suppressed the nitric oxide production in lipopolysaccharide activated RAW 264.7 cells in dose-dependent way through suppression of inducible nitric oxide synthase enzyme expression Decreased the Lipopolysaccharide-induced production of TNF-α in vitro and in vivo	Lee et al. (1999)
Dehydrocostus lactone derived from *S. lappa*	Pre-treated with (10 or 20 µM) for 1 h	RAW264.7 cells treated with Lipopolysaccharide (10 ng/ml) or MALP-2 (10 ng/ml) for an additional 8 h	Dehydrocostus lacton suppress the stimulation of nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3), the representative transcription factors included in the inflammation, stimulated by TLR agonists	Kim et al. (2020)

Table 2 (continued)

Bioactive compounds	Administration	Experimental design	Results	References
Sesquiterpene lactone (Costunolide) isolated from *Saussureae lappa* roots	Pre-treatment with 30, 50 µM for 1 h	Cultured cell lines (RAW264.7) enhanced with poly[I: C] (10 µg/mL) or Lipopolysaccharide (10 ng/mL) for 8 h	Prevented NF-κB stimulation and COX-2 induced by the TLR3 agonist polyriboinosinic polyribocytidylic acid (poly[I: C]) or the TLR4 agonist lipopolysaccharides	Park et al. (2009)
	Pre-treated with different level for 2h	Lipopolysaccharide -treated RAW 264.7 macrophages and U937 human monocytic cells	Effectively inhibited nitric oxide production by the inhibitory action on the inducible nitric oxide synthase gene expression by the suppression of NF-κB	Jin et al. (2000)
S. lappa marker compounds	Alantolactone, 5µM	HaCaT cells treated with TNF-α and IFN-γ (each 10 ng/mL)	In a dose-dependent manner, it supressed STAT1 phosphorylation in TNF-α and INF-γ-stimulated HaCaT cells	Lim et al. (2015)
Aqueous extract of root of *S. lappa*	Orally with a dose of (100, 200 and 300 mg/kg, p.o.)	Rats weighing 200–300 g injected with isoproterenol hydrochloride 85 mg/kg body weight	Significant action against isoproterenol-induced reduction of marker enzymes and oxidative stress in concentration-dependent way	Saleem et al. (2013)
Total methanol extracts of *S. lappa*	0.1 mg/ml	Lipopolysaccharide -stimulated peritoneal macrophages	Reduced more than 50% of cytokine induced neutrophil chemotactic factor (CINC) stimulation	Lee et al. (1995)

Table 2 (continued)

Bioactive compounds	Administration	Experimental design	Results	References
S. lappa ethanolic extract	oral administration for three weeks	Rats exposed to triamcinolone acetonide (TA)	Significantly reduced the production of pro-inflammatory cytokines (IL-12, and TNF-α) and C-reactive protein (CRP), and significantly boosted IgG and IgM compared to the control group	Abd El-Rahman et al. (2020a, b)
Saussurea lappa root extract	200 mg/kg body weight	Intraperitoneal injection for 28 days	Reduced lipid peroxidation and nitric oxide, and increased the glutathione activities	Abd El-Rahman et al. (2020a, b)
Saussurea lappa root extract	50 mg/kg body weight/day	Oral gavages for 4 weeks	Reduced all kidney toxicological signs induced by ethephon administration	Tousson et al. (2019)

application in shrimp (*Penaeus monodon*) cultured farms (Immanuel et al. 2004). To address these challenges, one potential approach is to employ suitable bio-encapsulation techniques to deliver the bioactive components to fish larvae. This method offers a potential solution for enhancing the efficacy and practicality of utilizing medicinal plant extracts in aquaculture settings. Artemia, which is a non-selective fish filter feeder, can be used as a bio-carrier to make the shrimp ingest essential nutrients and medicines (Immanuel et al. 2004, 2007).

In addition, while utilizing immunostimulants in aquaculture, it is important to consider the dosage, timing, manner of administration, and the fish's physiological status (Harikrishnan et al. 2011). Associations have been reported between the dose of immunostimulants and their effects, whereby an overdose may induce immunosuppression or other undesirable effects (Sakai 1999). However, in an acute toxicity study, an aqueous-methanol extract of *S. lappa* had a high safety margin when administrated at a high dose of 5 g/kg. It neither induced any behavioural changes nor mortality in mice (Yaeesh et al. 2010).

The cost-effectiveness and delivery methods of *S. lappa*

In last decades, the pharmaceutical sector has switched its major attention toward synthetic pharmaceuticals owing to their ease of quality assurance, low manufacturing cost, and time efficiency (Yaeesh et al. 2010). However, their expense, assurance, and effectiveness have always been questioned, leading to the reliance on plant-based medication development by more than 80% of the entire population in developed nations (Negi et al. 2014). The available literature demonstrated that the majority of the research concerned with the examination of the biological implications of the *S. lappa* extracts, which have been generated by common organic solvent extraction procedures (Yu et al. 2007). Thus, therapeutic techniques have been developed for employing nanotechnology to enhance drug delivery and establish innovative therapeutics (Tiwari et al. 2018). The anticipated advantages of encapsulating substances derived from medicinal plants were higher water solubility and bioavailability, protected medication against the environmental factors and a sustained administration (Madkour 2020). However, there is currently no proof of the implications of encapsulating both the extract and the EO of the *S. lappa* in fish diets (as immunostimulants) or for disease treatment. Though, Lammari et al. (2021) recently demonstrated that the anti-Alzheimer, anti-inflammatory, and antidiabetic features of the extracted *S. lappa* essential oil employing costunolide (8.87%) and dehydrocostus lactone (55.39%) as the main molecules are further improved by its encapsulation in polymethyl methacrylate-based nanoparticles (approximately 145 nm size), as well as a good stability after 30 days storage at different pH levels and temperature degrees. In addition, innovative technology in plant extract creation depends on an efficient use of the methods of extraction, involving solvents. Methanol, acetone, and ethanol are frequently used as solvents, however hydroextraction seems to be more favourable in terms of potential ecological impacts. The correct solvent is also very important for the functional characteristics of *S. lappa*, which may display antifungal or antibacterial activity in addition to the immune stimulating influence (Abd El-Rahman et al. 2020a, b).

Biological activities

Antibacterial activity

Medicinal plants and their medicinal preparations have been identified to have effective antimicrobial properties that provide protection to the host against undesirable results of infectious diseases (Tiwari et al. 2018). The ethanol extract of *S. lappa* has been reported to exhibit antibacterial action in a dose-dependent manner against *Streptococcus mutans*. It showed characteristic suppression at amounts higher than 0.5 mg/ml as compared to the control group (Yu et al. 2007).

S. lappa extract contains active ingredients, such as costunolide and dehydrocostus lactone, which have antibacterial properties (Negi et al. 2014). Methanol extracts of *S. lappa* have been examined for their antibacterial activity against gram-negative and gram-positive bacteria, where the results showed their suppressive action on different bacterial species with minimum inhibitory concentration (MICs) ranging from 3.12 to 12.50 µg/µl for *Escherichia coli* and *Citrobacter freundii*, from 6.25 to 25.00 µg/µL for *Enterococcus faecalis*, and from 6.25 to 50.00 µg/µl for *Staphylococcus aureus* (Negi et al. 2014). The authors found that samples of *S. lappa* containing higher contents of costunolide and dehydrocostus lactone showed higher antibacterial activity. The main phase in the cytoplasmic biosynthesis of peptidoglycan precursors of the bacterial cell wall depends on the MurA enzyme (O'Shea 2016).

The mechanism of action of some sesquiterpene lactones as antibacterial agents exploits the effect of cynaropicrin on MurA of *E. coli* (Bachelier et al. 2006). Cynaropicrin has an unsaturated ester side chain, which is very important for the irreversible blocking of MurA. Therefore, the sesquiterpene lactones target MurA to trigger their significant antibacterial action (Bachelier et al. 2006) (Fig. 1).

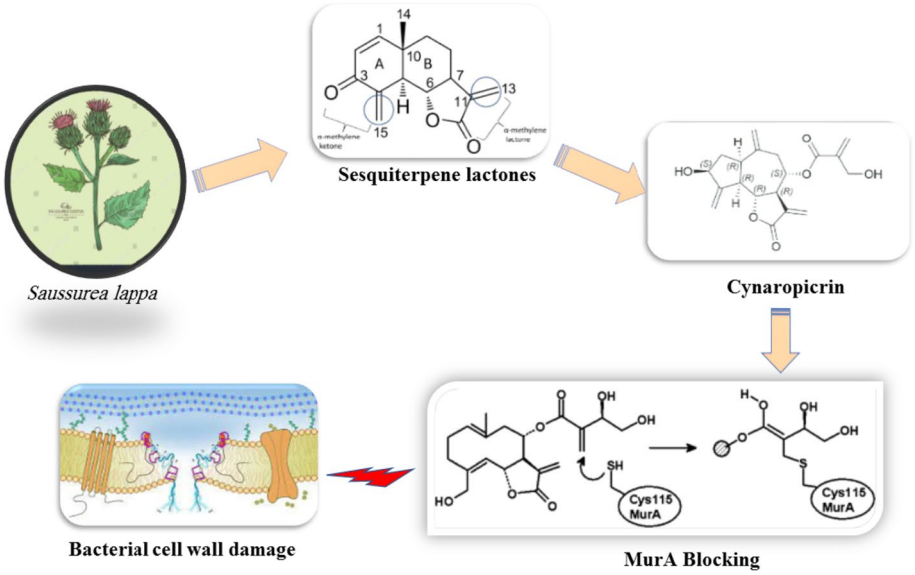


Fig. 1 The antibacterial mode of action of *Saussurea lappa*

An intriguing finding reveals that *Helicobacter pylori*, a prevalent human pathogen associated with severe clinical manifestations, has been detected in both wild and cultured tilapia from various regions. This suggests that tilapia could potentially serve as a zoonotic carrier of *H. pylori*, potentially designating it as a new fish-borne pathogen (Abdel-Moein et al. 2015). Among the ethanol and aqueous extracts of 30 medicinal plants, ethanol extract of *S. lappa* showed the third most anti-*H. pylori* activity with the MIC of ~40.0 µg/ml when studied for in vitro antibacterial action using six different strains (Li et al. 2005). The anti-*H. pylori* effect of *S. lappa* appears to be attributable to its volatile oils. Essential oils are known to possess in vitro antibacterial effects against *Pseudomonas* spp. (Kačániová et al. 2017), *Streptococcus agalactiae,* and *Aeromonas hydrophila* (de Souza Silva et al. 2019) isolated from Nile Tilapia fish, which may be attributed to the presence of dehydrocostus lactone and costunolide. The antibacterial activity of these two agents is very potent in pharmaceutical research (Choudhary et al. 2016).

Recently, some opportunistic bacterial fish pathogens, such as *Enterococcus faecalis,* have been highlighted as the cause of various epidemics in different aquatic farms, which led to mass mortalities in various fish species (Rahman et al. 2017). However, *Enterococcus*-resistant strains do not respond to different families of antibiotics (Arias and Murray 2012).

The antibacterial properties of several medicinal plant extracts, including *Acacia modesta*, *A. absinthium*, *Nigella sativa*, and *S. lappa*, were examined against two gram-negative bacteria, *Salmonella typhi* and *Pseudomonas aeruginosa*, as well as three gram-positive bacteria, *Staphylococcus aureus*, *E. faecalis*, and *Bacillus subtilis*. The results demonstrated that the extracts of *S. lappa* exhibited remarkable antibacterial activity against all five bacterial strains (Khalid et al. 2011). Additionally, among all the medicinal plants evaluated, both *S. lappa* and *N. sativa* extracts displayed the strongest antibacterial effects (Khalid et al. 2011).

Antiparasitic activity

In recent years, the issues arising from parasitic infestation in aquariums have garnered significant attention due to the economic losses associated with fish mortality. Moreover, these infestations negatively impact factors such as body mass, behavior, resistance to environmental stressors, and fish susceptibility to predation (Scholz 1999). *Trypanosoma* is an extracellular protozoan, whose presence in fish blood causes 100% fish mortality (Xie et al. 2019).

Different studies have recently recorded that sesquiterpene lactones show special anti-trypanosomal activity to overcome trypanosomal infestation (Julianti et al. 2011). The ethyl acetate extract of *S. lappa* roots was estimated for its anti-trypanosomal activity, which potently inhibited *Trypanosoma brucei* rhodesiense by 96% at a dose of 4.8 µg/ml (Julianti et al. 2011). There are numerous biological actions of sesquiterpene lactones that occur through the reaction of their α-methylene-γ-butyrolactone functionality with the thiol groups of biomacromolecules through the Michael addition reaction (Julianti et al. 2011).

Forty sesquiterpene lactones from five sesquiterpene lactone classes were studied in vitro for their anti-trypanosomal activity. The results confirmed the impact of the α-methylene-γ-butyrolactone fraction on their cytotoxic influence, in addition to the anti-trypanosomal effect (Julianti et al. 2011).

Cynaropicrin, a sesquiterpene lactone, is the first herbal-origin natural product with an anti-trypanosomal effect, which is enhanced by the exhaustion of intracellular

trypanothione (TSH)2 and glutathione (GSH) and the suppression of trypanosomal ornithine decarboxylase (Elsebai et al. 2016). Different sesquiterpene lactones are an effective therapeutic strategy to enhance their efficiency and decrease their side effects. These combinations may have an additive antiparasitic effect as a result of the interactions with several targets (Sülsen et al. 2016). The high incidence of *Clonorchis sinensis* infection in freshwater fish, especially in some parts of Asia, is a major public health concern (Chen et al. 2010; Zhang et al. 2014b).

This fish-borne zoonotic fluke is a common cause of human diseases caused by trematodes (Chai et al. 2005). This fluke is transmitted actively along rivers, especially putting those individuals at risk who routinely eat raw or undercooked fish. Moreover, the infestation has been recorded as a predisposing cause for perihilar cholangiocarcinoma, which is ranked as the second most common primary malignant tumour of the liver after hepatocellular carcinoma (Zhang et al. 2014b). *S. lappa* has demonstrated antiparasitic properties against *C. sinensis*, a parasitic flatworm, as well as certain nematode infections (Rhee et al. 1985). When orally administered to rabbits infected with *C. sinensis*, *S. lappa* exhibited a suppressive effect on the pathogen's egg-laying capacity (Rhee et al. 1985). Additionally, in a field trial involving naturally infected children, the anti-nematodal activity of *S. lappa* was observed (Akhtar and Riffat 1991).

Anti-tumour activity

Recently, sesquiterpene lactones were observed to have powerful anti-cancer activities through numerous signalling pathways, including reactive oxygen species (ROS) generation (Rasul et al. 2013), c-Jun N-terminal kinase (JNK) stimulation (Choi and Lee 2009), NF-κB (Wang et al. 2017), and telomerase activity suppression (Kanno et al. 2008). Sesquiterpene lactones exhibit anti-tumour action on IKKβ, leading to the inhibition of the NF-kB signalling pathway (Kim et al. 2020). The cytotoxic activity of *S. lappa* root extract against A549 and C-6 cells was high, suggesting that *S. lappa* roots could be a rich source of anti-cancer agents (Kumar et al. 2014).

The bioactive phytoconstituents derived from *S. lappa* play a role in various crucial biological processes, including cell cycle arrest, apoptosis, and DNA repair (Rasul et al. 2013). Furthermore, they are involved in the assembly of microtubule proteins and the inhibition of telomerase activity (Lin et al. 2015). Robinson et al. (2008) discovered a novel sesquiterpene lactone with anti-cancer properties when tested on challenging cell lines. Additionally, they investigated the anti-cancer activities of costunolide and its derivatives, determining them to possess moderate anti-cancer activity. Costunolide, a sesquiterpene lactone, is extracted from the roots of *S. lappa*, and has potent anti-cancer action against different kinds of cancer (Lee et al. 2011).

The anti-cancer effect of costunolide was studied in vitro by analyzing cell viability and apoptosis in human bladder cancer cells (T24 cells). The results revealed inhibition of the proliferation of T24 cells and enhanced cell death through stimulation of ROS-mediated apoptosis, which stimulated mitochondrial permeability transition (MPT) and cytochrome c release into the cytosol (Rasul et al. 2013). Examination of the apoptosis-related proteins in T24 cells showed that costunolide enhances the expression of Bax with parallel suppression of Bcl-2 and survivin expression, which results in the dissipation of mitochondrial membrane potential ($\Delta\Psi m$) and the subsequent stimulation of caspase-3 and its downstream substrate, PARP, ending in apoptosis (Rasul et al. 2013).

In cells treated with costunolide, pre-treatment with an antioxidant, such as N-acetyl cysteine, significantly blocks these effects (Lee et al. 2001). Costunolide has been reported to play a role in initiating apoptosis and its putative pathways of action through the mitochondria-dependent pathway in SGC-7901 cells by stimulating caspase-3, downregulating Bcl-2 (Rasul et al. 2012), and upregulating Bax (Rasul et al. 2013).

To investigate the mechanism underlying costunolide-induced apoptosis in human prostate cancer PC-3 cells, multiple pharmacological and biochemical analyses were conducted. The findings demonstrated that costunolide triggers a rapid increase in nuclear Ca^{2+} levels and induces phosphorylation of the mutated ataxia telangiectasia and Rad3-related (ATR) protein kinase. The activation of ATR kinase serves as a signaling marker in response to DNA damage (Hsu et al. 2011). Costunolide also stimulates G1-phase cell cycle arrest, which is then maintained by p21 upregulation, and its relationship with the cyclin-dependent kinase 2/cyclin E complex, leading to the stop of G1 of the cell cycle and apoptotic cell death in human prostate cancer cells (Yu et al. 2007). The release of intracellular Ca^{2+} potentiates ROS production, which leads to Ca^{2+} accumulation and stimulation of p38, JNK, and ERK1/2 (Hung et al. 2010).

The majority of proteins secreted or displayed on the cell surface enter the endoplasmic reticulum (ER) first, where they are folded and brought together (Walter and Ron 2011). Toxic by-products, such as Ca^{2+}, along with failed protein synthesis, folding, transport, or degradation, can disrupt the ER function and lead to ER exhaustion, enhancing various definite signalling pathways involved in apoptosis (Lin et al. 2015).

Cytostatic actions of *S. lappa* extracts were also observed using gastric AGS cancer cells. The results showed that treatment with *S. lappa* extracts induced apoptosis and G2 arrest in a time and concentration-dependent way (Ahn et al. 1998). Extracts of *S. lappa* can produce anti-cancer effects by modulating cyclins and pro-apoptotic compounds and suppressing anti-apoptotic compounds (Ko et al. 2005). Hence, the results suggest that *S. lappa* extracts have the potential to serve as preventive and therapeutic agents for gastric cancers by incorporating them as medicinal and herbal supplements in functional animal feed (Lin et al. 2015).

In addition, the anti-carcinogenic activity of cynaropicrin, extracted from *S. lappa*, was assessed against various cancer cells derived from leukocytes, as well as other cell types such as fibroblasts and Chang liver cells (Secombes et al. 1996). Cynaropicrin, a sesquiterpene lactone extracted from *S. lappa* root, exerts anti-carcinogenic activity (Kang et al. 2004). Cynaropicrin shows potent inhibition of leukocyte cancer cell lines, such as U937, Eol-1, and Jurkat T-cells in a time-dependent manner, while it weakly inhibits the propagation of some other cancer cells, such as Chang liver cells and human fibroblast cell lines (Cho et al. 2004b).

These observations suggest that cynaropicrin may stimulate apoptosis activation and cell death by stimulating caspase-dependent apoptosis, leading to cell cycle arrest and DNA fragmentation (Cho et al. 2004b).

Anti-inflammatory activity

Acute inflammation is a potential signal of innate immunity that enhances the elimination and healing of infected or injured tissues. However, chronic inflammation contributes to several diseases (Rodgers et al. 2020). Inflammation denotes the activation of vigorous uncontrolled immune responses. NF-κB controls the transcription of genes involved in innate and adaptive immune responses. The activation of NF-κB is a crucial process that

mediates inflammatory and stress responses, establishing a connection between chronic inflammation, apoptosis, and cancer (Caamano and Hunter 2002). NF-κB serves as a key inducer of multiple pro-inflammatory gene expressions and plays a regulatory role in the survival, activation, and differentiation of innate immune cells and inflammatory T-cells (Liu et al. 2017).

Costunolide, a common sesquiterpene lactone that exists in numerous medicinal plants, is observed to have a different biological activity (Park et al. 2009). In a luciferase reporter gene assay, costunolide isolated from *S. lappa* inhibited NF-κB stimulation and cyclo-oxygenase-2 expression (COX-2) stimulation by poly [I: C] (TLR3 agonist) or LPS (TLR4 agonist) in a concentration-dependent way (Shin et al. 2012). The disturbance of TLR signalling pathways results in different chronic inflammatory disorders (Kim et al. 2020).

Dehydrocostus lactone was studied for its effect on NF-κB stimulation enhanced by LPS (TLR4 agonist) or MALP-2 (TLR2 and TLR6 agonist) by NF-κB luciferase reporter gene assay. This experiment revealed that the activation of NF-κB was inhibited more strongly by LPS and MALP-2 (Kim et al. 2020). NF-κB stimulation in macrophages controls various important genes, including COX-2. LPS or MALP-2 enhance its expression in RAW264.7, while it is suppressed by dehydrocostus lactone (Kim et al. 2020). The expression of COX-2 is enhanced by LPS but is suppressed by *S. lappa* extracts via the downregulation of NF-κB, mitogen-activated protein kinases (MAPKs), and activator protein 1 (AP-1) via MyD88 signalling (Chun et al. 2012). Sixteen sesquiterpenoids, including two new compounds, namely, saussucostusosides A and B, were recently extracted from *S. lappa* roots. Among the extracted compounds, costunolide, 3b-[4-hydroxymethacryloyloxy]-8a-hydroxycostunolide, and 11b,13-dihydrozaluzanin C potently suppressed LPS-induced NO generation in RAW264.7 cells (Hanh et al. 2021).

In salmon, the use of soybean meal in ration formulation is limited due to its potential to activate the gut defence system, leading to the development of inflammation-like conditions in the distal part of the intestine, known as enteritis. This condition is characterized by inflammatory infiltration in the mucosa, reduced epithelial vacuolization, and atrophy of primary and secondary mucosal folds (Thorsen et al. 2008). Accordingly, dose-dependent negative effects of soybean meal were noticed on nearly all performance parameters and enzyme activities with increasing soybean meal inclusion in Atlantic salmon (Krogdahl et al. 2003).

Proteinase-activated receptor 2 (PAR-2) promotes inflammation in the intestinal mucosa of mammals and is enhanced by trypsin and other serine proteinases (Thorsen et al. 2008). PAR-2 is involved in the G protein-coupled receptor subfamily and is involved in the homeostasis of epidermal permeability barrier function (Yang et al. 2017). Thus, PAR-2 activation, through IKKβ activation, is an important part of the pathogenesis of different digestive disorders, leading to DNA binding and nuclear translocation of NF-kB. However, extracts of *S. lappa* root have been recorded to decrease inflammation induced by various stimulants in different cells and tissues (Lim et al. 2014). The upregulation of PAR-2 increases the stimulation of intercellular adhesion molecule 1 (ICAM-1) and interleukin-6 (IL-6), resulting in the over-stimulation of inflammation and immune responses (Yang et al. 2017). An extract from *S. lappa* was able to decrease the content of proinflammatory cytokines, which led to the prevention of an exaggerated immune response. In contrast, the combination of *S. lappa* and *Thuja orientalis* L. can reduce inflammation by reducing PAR-2 expression (Yang et al. 2017).

Macrophages play a vital role in immune response and inflammation. Their activity is modulated by various cytokines and inflammatory mediators, including interleukin-1β (IL-1β), prostaglandins, TNF-α, and nitric oxide. IL-1β, primarily produced by monocytes and

macrophages, is a pro-inflammatory cytokine with diverse functions in the inflammatory process (Kang et al. 2004). Costunolide shows an inhibitory effect on IL-1β gene expression in macrophages by suppressing the activity of MAPKs and the DNA binding of AP-1 in LPS-stimulated RAW 264.7 cells (Kang et al. 2004).

Any experimental studies have indicated that alleviating macrophage cytokines is an important part of the anti-inflammatory response (Kang et al. 2018). TNF-α is one of the main pro-inflammatory cytokines that enhances the production of other pro-inflammatory cytokines, including IL-6 and IL-1β (Kang et al. 2018). Activated macrophages produce TNF, while neutrophils produce TNF, which quickens tumorigenesis (Taniguchi and Karin 2018). Sesquiterpene lactones extracted from *S. lappa* have been found to show immunomodulatory activities that help control the function of macrophages and monocytes via the production of TNF-α and nitric oxide (NO) (Cho et al. 1998). Sesquiterpene lactones, including costunolide and dehydrocostus lactone, were found to inhibit the stimulations of NF-κB and inducible nitric oxide synthase (iNOS) (Matsuda et al. 2003; Choi et al. 2012).

Besides sesquiterpene with an α-methylene-γ-butyrolactone moiety, the amino acid-sesquiterpene conjugates (saussureamines A and B) extracted from *S. lappa* have shown potential suppressive action on NO production in LPS-activated macrophages (Matsuda et al. 2003). Moreover, cynaropicrin strongly inhibits cytokine production, such as TNF-α, at non-cytotoxic concentrations, as well as inhibits cytokine-induced neutrophil chemoattractant-1 (IL-8) and the release of NO (Cho et al. 2000). Extracts from *S. lappa* exhibit inhibitory action on chemokine production stimulated by TNF-α and IFN-γ in the HaCaT human keratinocyte cell line. This effect may be due to the potential of *S. lappa* to inhibit the phosphorylation of signal transducer and activator of transcription (STAT1) (Lim et al. 2015).

Inflammation is significantly influenced by the upregulation of adhesion molecules in response to various inflammatory factors (Ishikane et al. 2018). These molecules play a critical role in immune cell regulation within inflamed tissues and facilitate cell–cell adhesion. Therefore, effectively blocking these events is considered a promising strategy and therapeutic target for suppressing chronic inflammation (Cho et al. 2004a). Extracts from *S. lappa* control the functional stimulation of major adhesion molecules (CD29 and CD98) in macrophages; for instance, cynaropicrin blocks CD29- and CD98-induced U937 homotypic aggregation at non-cytotoxic concentrations by disturbing extracellular signal-related kinase (ERK) stimulation. Furthermore, cynaropicrin can be used to inhibit CD29- and CD98-mediated disorders, such as invasion, migration, and metastasis of leukocyte cancer cells and virus-induced chronic inflammation (Cho et al. 2004a).

As previously mentioned, the significance of sesquiterpenoids derived from *S. lappa* is increasingly acknowledged for their ability to inhibit pro-inflammatory enzymes and mediators (Laher 2014). Several studies have proposed potential mechanisms to explain these effects. One study suggests that sesquiterpene lactones isolated from *S. lappa* may exert their anti-inflammatory activity in macrophages by inducing the expression of heme oxygenase-1 (HO-1) through a distinct mechanism (Choi et al. 2012).

Therefore, the inflammatory response is a complex process involving multiple factors acting in concert to produce an inflammatory response to the invasion by viruses, parasites, and bacteria, part of the cell wall structure of gram-negative bacteria, some other pro-inflammatory agents that induce changes involved in inflammation, and particularly LPS (Laher 2014).

Administration of LPS to RAW264.7 cells for 1 h caused phosphorylation and degradation of IκB-α, an inhibitor of NF-κB nuclear translocation. Pre-treatment with sesquiterpene lactone for 12 h significantly suppressed LPS-induced phosphorylation and

degradation of IκB-α (Choi et al. 2012). Furthermore, an increased level of nuclear p65 protein, as a response to LPS stimulation, was reported to be inhibited with increasing levels of sesquiterpene lactone (Choi et al. 2012).

Antioxidant activity

Oxidative stress in fish has long been a topic of significant interest in various fields, including environmental and aquatic toxicology (Naiel et al. 2020). Various chemical compounds such as pesticides (Naiel et al. 2020) and heavy metals (Begam and Sengupta 2015) can induce oxidative stress. Therefore, the presence of pro-oxidant agents in fish can be used as an indicator to assess pollution levels in specific areas or globally (Slaninova et al. 2009). Furthermore, different environmental pollutants have the potential to disrupt the normal function of macrophages and impair their phagocytic capacity, leading to compromised immune responses in aquatic organisms (Begam and Sengupta 2015).

On the other hand, circulating phagocytes, which constitute a non-specific immune response, are very important in the immunological response of fish against invading pathogens (Fatima et al. 2000). However, many cell types can engulf particulate agents; "professional phagocytes" perform this function much more effectively than other cells because of the expression of unique membrane proteins and signal transduction mechanisms in them (Brown 1992). As in humans, pathogen recognition via receptors of the innate immune system, such as TLRs and nucleotide-binding and oligomerisation domain (NOD)-like receptors (NLRs), activates a range of inflammatory responses (Tschopp and Schroder 2010). Invading foreign pathogens are eliminated via activated fish phagocytes that damage the pathogens by releasing cytotoxic ROS (Neumann et al. 2001).

ROS play a major role in mediating intracellular signalling cascades and can eliminate pathogens directly by enhancing oxidative destruction of bio-compounds or indirectly via stimulating pathogen destruction by different non oxidative activities, including autophagy, pattern recognition receptors signalling, T-lymphocyte response, and neutrophil-based extracellular trap formation (Paiva and Bozza 2014). Therefore, it should be expected that the reduction of ROS production enhances infection. Growing evidence supports that in certain disorders, prooxidants elevate the pathogen burden and decrease antioxidants (Paiva and Bozza 2014). A large amount of ROS is produced by triggered phagocytes or by other stressors that can produce extensive cellular damage and apoptosis (Slater et al. 1995; Fatima et al. 2000).

ROS is also implicated in lipid peroxidation and/or intracellular thiol depletion, leading to mitochondrial dysfunction and cell death (Vercesi et al. 1997). In addition, ROS production by stimulated phagocytes links organ-specific peroxidative damage in fish (Santos et al. 2006).

S. lappa, an antioxidant-rich medicinal plant, plays a crucial role in reducing oxidative stress by eliminating excessive free radicals (Choi et al. 2009; Saleem et al. 2013; Benedetto et al. 2019; Abd El-Rahman et al. 2020a, b). Among the various solvent fractions derived from *S. lappa* roots, the n-butanol-soluble fractions (at 1,000 ppm) demonstrated a suppressive effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and exhibited a reduced power of 92.98% and 0.38, respectively. These values were comparable to those of the ascorbic acid positive control (Chang et al. 2012). However, even at higher concentrations of *S. lappa*, cell toxicity was observed for some fractions (Chang et al. 2012), indicating that *S. lappa* roots can be used as an antioxidant mediator in a functional fish diet.

Moreover, the antioxidative activities of *S. lappa* in both test systems were elevated in a dose-dependent manner (Chang et al. 2012),

Oxygen radicals are a major cause of oxidative modification of proteins that may cause disturbance in biological functions and cell death (Redza-Dutordoir and Averill-Bates 2016). Carbonyl formation is a type of oxidative change in amino acid residues of proteins, and its increased levels have been detected in several ailments (Reznick and Packer 1994). It has been recorded that dehydrocostus lactone has an inhibitory effect on H_2O_2-induced protein oxidation (Choi et al. 2009). They also studied the antioxidant effect of dehydrocostus lactone (0.1–10 µg/ml) on osteoblastic MC3T3-E1 cells, which were incubated with 0.3 mM H_2O_2. The results showed that the treatment with 10 µg/ml dehydrocostus lactone minimized the production of osteoclast differentiation-inducing factors, such as interleukin (IL)-6, and the receptor activator of NF-κB ligand (RANKL) in the presence of H_2O_2 (Choi et al. 2009). Furthermore, dehydrocostus lactone (0.4–2 µg/ml) inhibited the formation of protein carbonyl (PCO), and malondialdehyde (MDA) stimulated by H_2O_2 in osteoblasts (Choi et al. 2009). It is thus suggested that dehydrocostus lactone can inhibit oxidative damage and H_2O_2-induced cellular dysfunction. Essential oils extracted from roots of *S. lappa* expressed the highest antioxidant activity; the greatest amount of the main biological compound, dehydrocostus lactone, was found in 3-year-old plants collected during the summer (Benedetto et al. 2019).

In rats, the hot water extract of *S. lappa* demonstrated a concentration-dependent amelioration of oxidative myocardial injury induced by isoproterenol (Saleem et al. 2013). Abd El-Rahman et al. (2020a, b) suggested that *S. lappa* could serve as a potent natural antioxidant to counteract the adverse effects of glucocorticoids. The study confirmed that the ethanolic extract of *S. lappa* could alleviate the oxidative and apoptotic effects of triamcinolone acetonide in rats (Abd El-Rahman et al. 2020a, b).

An enhancement of antioxidant enzymes was found when an aqueous extract of *S. lappa* (300 mg) was administered to deltamethrin-intoxicated animals (Alnahdi et al. 2016). Administration of 600 mg of *S. lappa* ethanolic extracts for three weeks to normal animals produced a marked increase in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the spleen and lung as compared to the control group (Abd El-Rahman et al. 2020a, b). However, a marked reduction in the lipid peroxidation marker, MDA, was also found in animals that received *S. lappa* as compared to the control. Moreover, *S. lappa* administration, either previously or concurrently in TA-exposed rats, reversed the restoration of antioxidant enzyme action and inhibited lipid peroxidation (Abd El-Rahman et al. 2020a, b). Numerous pieces of evidence suggest that *S. lappa* is rich in flavonoids and phenolic compounds, which are known for their antioxidant properties (Saleem et al. 2013).

Anti-complementary activity

The complement system, an ancient guard against pathogens, is an important arm of innate immunity (Ellis 2001) and is one of the major effective responses of antibody-mediated immunity (Walport 2001). The complement system contains more than 30 soluble plasma proteins and cell surface receptors that serve as a vital humoral system of unspecific immunity and a link between specific and non-specific immune responses (Boshra et al. 2006; Huang et al. 2019). It comprises different common activation pathways, including classical, lectin and alternative pathways (Walport 2001).

The complement regulatory systems are well balanced. When this delicate equilibrium is disrupted, it can lead to self-attack and a variety of diseases (Walport 2001). Functional

and structural studies have revealed that sulphated polysaccharides are the best alternatives for therapeutic purposes if the complement system is overactive, because a disruption between complement activation and inhibition can cause significant cell damage (Wang et al. 2012; Jiang et al. 2015; Zhang et al. 2015; Huang et al. 2019).

Five sulphated by-products, extracted and purified from *S. lappa* root, have been identified with varying degrees of sulphation ranging from 0.40 to 2.18. These compounds have demonstrated a strong anti-complement effect, affecting both the classical and alternative pathways, in a concentration-dependent manner (Fan et al. 2014). Additionally, they exhibited limited anticoagulant activity (Fan et al. 2014). The findings indicated that a higher degree of sulphation correlated with an enhanced anti-complement effect. However, further research is needed to fully explore the extraction and bioactivity of polysaccharides from costus root and their potential benefits (Fan et al. 2014).

Immunomodulatory role of *S. lappa*

The fish immune response has been reported to be similar to that of humans (Iwama et al. 1997). However, important differences exist between the immune system of fish and mammals, including the absence of lymph nodes and bone marrow in fish. The kidney, spleen and thymus are the main lympho-myeloid tissues of the teleost species, while the skin, intestine, and liver are also important factors involved in the defence system (Dalmo et al. 1997).

Teleost fish, the largest and most varied vertebrates, have phagocytic cells, such as neutrophils, natural killer (NK) cells, macrophages, and T and B lymphocytes. Teleost fish also exhibit several humoral immune responses that employ natural hemolysin, C-reactive proteins, lysozyme, and transferrin. These responses complement the classical and alternative immune pathways. The presence of cytokines (interferon, interleukin 2, macrophage activating factors) has also been reported (Sakai 1999).

Phagocytes play a crucial role in the innate immunity of fish (Kiron 2012). Understanding the modulation of phagocyte function, including their activation by various molecules and inhibition by others, is essential for achieving protective responses by controlling phagocyte activity (Secombes and Fletcher 1992). The activation of macrophages has an immense capability to destroy microorganisms, including bacteria, such as *Renibacterium salmoninarum* and *Aeromonas salmonicida*. Activated macrophages in trout release factors that have a positive autocrine activity on effector functions, such as the production of bactericidal ROS (Secombes et al. 1996).

Immunostimulants extracted from medicinal plants increase host defence against infection by stimulating specific and non-specific defence systems. They are used extensively to improve the weakened immune responses in fishes (Harikrishnan et al. 2011). Furthermore, immune suppression caused by sex hormones can be reduced by immunostimulants (Sakai 1999).

Polysaccharides from numerous common medicinal plants have been proven to be immune-potentiating either in vitro or in vivo (Harikrishnan et al. 2011). Sulphated polysaccharides from *S. lappa* roots can enhance the release of different cytokines (Ponce et al. 2020) and produce antibodies to stimulate the complement system (Huang et al. 2019).

In addition, the regulation of the function of macrophages, such as T/B lymphocytes and NK cells, can be affected by sulphated polysaccharides that exert their immunological activities on them (Huang et al. 2019). Gene expression profiles from a recent study

showed that intraperitoneal injection of a sulphated polysaccharide, ulvan, extracted from *Ulva ohnoi* in Senegalese sole juveniles stimulated many signalling pathways of the immune system in various tissues. Moreover, the immune response pathways could be modulated via sulphated polysaccharides in response to *Photobacterium damselae* sub sp. *piscicida* challenge, indicating that these polysaccharides may possibly substitute as nutraceuticals and/or vaccine adjuvants in aquaculture (Ponce et al. 2020).

Sulphated polysaccharides exhibit immunological effects that are dependent on the source of the polysaccharide and their structural characteristics, such as the degree and molecular weight of sulphation. However, further research is needed to understand the relationship between immunological responses and the structural properties of sulphated polysaccharides (Huang et al. 2019).

Costunolide has immunosuppressive activities. Previous studies have shown that costunolide prevents the destructive action of cytotoxic T lymphocyte (CTL) by suppressing the increment in tyrosine phosphorylation in response to the crosslinking of T-cell receptors (Taniguchi et al. 1995). Yuuya et al. (1999) examined more than 100 medicinal plants to find a component that reduces the destructive activity of CTL. The results showed that only *S. lappa* extracts had considerable inhibitory activity, which appeared to take place by binding the bioactive components with the cysteine residues of tyrosine kinase, hindering kinase activity in T-cells (Yuuya et al. 1999). These immunostimulants mainly improve the function of phagocytes and humoral immune responses. The stimulation of these immunological responses depends on increased resistance and protection against infectious diseases (Sakai 1999).

In particular, compared to synthetic pharmaceuticals, syrigaresinol, which was isolated from *S. lappa*, has remarkable antiviral features. Meanwhile, it seems that this syrigaresinol compound may be examined to see whether it is capable of combating the Covid-19 based on the energy binding score (Prawiro et al. 2021). As a result, it is possible that *S. lappa* will be developed as an effective antiviral medicine in the near future (Vincent et al. 2020). Several studies have also revealed that flavonoid molecules found in certain medicinal plants have a high docking affinity against viral infectious agents (Jo et al. 2020). In addition, other flavonoid glycosides including neoeriocitrin, isonaringin, hesperidin isolated from *Saussurea* spp. roots have revealed many antiviral properties (Tousson et al. 2019). The hesperidin anti-viral features might be related with boosting host cellular immunity against viral infection and its effective anti-inflammatory properties might assist in modulating cytokine pathways (Haggag et al. 2020). The immunostimulatory mechanisms of *S. lappa* are depicted in Fig. 2.

Health and conversion concerns of *S. lappa*

S. lappa is regarded as the most indigenous and widely used medicinal plant in Pakistan. Additionally, it is frequently used in traditional and herbal medicine, as well as in novel synthetic medications. In India, throughout 2014 and 2015, the medical sectors utilized around 164.65 MT of *S. lappa*. Because of huge request, most native populations of certain species have either been disappeared or are subjected to aggressive harvesting, therefore the availability of this vital medicinal plant in the wild is declining daily. The wild *S. lappa* is indigenous to a geographically restricted region of the Himalayas and develops on wet mountains at elevations of 2600 up to 4000 square meters (Amara et al. 2017). Aside of the restricted distribution, one of the reasons for the threat is the collection of the whole

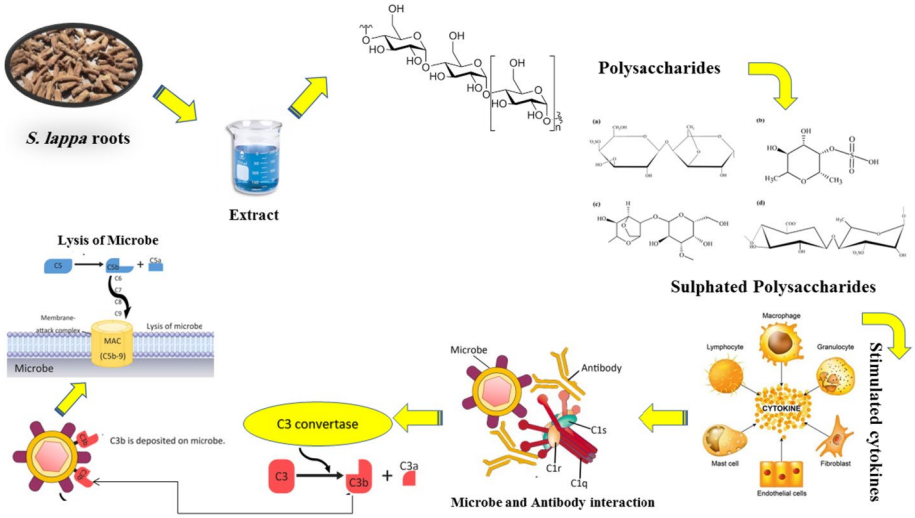


Fig. 2 The immunostimulatory mechanisms of *Saussurea lappa*

plant for personal use. Thus, *S. lappa* is regarded one of the 37 Himalayan rare herbs that have been organized for in situ and ex situ protection regulation (Chang et al. 2012). Furthermore, in order to fill the gap between the expanding demand for *S. lappa* and the pharmaceutical business, commercial cultivation is currently being carried out on a larger scale. China was classified as the greatest exporter of *S. lappa*, having exported 1024 tons during 1983 to 2009, while India was the second-highest supplier (Rathore et al. 2021). Therefore, it is essential to focus on the conventional understanding of local populations, encourage their efforts to increase and preserve the medical wealth, and impose their recognition of the variables influencing the herbs and their inspection of the state of preservation of herbs, as well as avoid harvesting the whole plant for personal use.

In contrast, several reports documented *S. lappa* pharmacological advantages but did not provide enough information to assess its detrimental consequences. For example, Ansari (2019) found that concentrated extract of the root taken in higher dosages for long term generated various undesirable effects such as cerebral centres depression, gastrointestinal disorders, chronic headache, urethral irritation, and giddiness. Until recently, the harmful effect of long-term use of *S. lappa* in fish therapy has not been adequately explored and requires additional detailed investigation.

Concluding remarks

S. lappa, also known as burdock, is a widely recognized medicinal plant in many countries and is utilized for the treatment of various human diseases. The therapeutic potential of *S. lappa* is attributed to its bioactive compounds, particularly sesquiterpene lactones such as costunolide and dehydrocostus lactone. These compounds exhibit a wide range of biological activities, including anti-cancer, anti-inflammatory, hepatoprotective, anti-ulcer, cholagogic, anticonvulsant, immunomodulatory, gastroprotective, angiogenesis, hypoglycemic,

spasmolytic, anti-hepatotoxic, antidiarrheal, hypolipidemic, antiparasitic, antimicrobial, and antiviral properties.

Recent studies have shown that the preventive use of *S. lappa* yields better outcomes than using it as a treatment, particularly in cancer therapy. The plant has been demonstrated to possess antioxidant, anti-apoptotic, and anti-inflammatory properties against pesticide pollution. The methanolic extract of *S. lappa* has been found to inhibit the production of TNF-α, a pro-inflammatory cytokine, in murine macrophage-like cells. Further investigation led to the identification of three sesquiterpene lactones (reynosin, cynaropicrin, and santamarine) that exhibit a dose-dependent suppression of TNF-α synthesis. Certain chemical components of *S. lappa* show potential for transformation into bioactive molecules.

For instance, compounds like costunolide and cynaropicrin have been identified as inactive anti-cancer agents. Cynaropicrin, in particular, has been recognized as a primary inhibitor of TNF-α. However, it is important to note that while there is some evidence supporting the safety and potential efficacy of *S. lappa*, the quality of the available evidence is low. Many aspects related to its active components, physiological routes, pharmacokinetics, bioavailability, and impact on consumer health are not yet thoroughly understood. As *S. lappa* holds various therapeutic uses in veterinary medicine, further in vitro, in vivo, and pathological research is needed to explore its potential for promoting sustainability in aquaculture. Overall, *S. lappa* shows promise as a medicinal plant with diverse therapeutic applications, but more comprehensive and high-quality research is necessary to fully understand its mechanisms of action, life cycle production assessment, production sustainability approaches and potential benefits in both human, ecological impacts and veterinary medicine.

Author's contribution All authors contributed to the study conception and design. Acquisition and data collection were performed by Abdelrazeq Mohamed Shehata, Vinod Kumar Paswan, Mourad Ben Said, Khaled A. El-Tarabily. The first draft of the manuscript was written by Mohammed Abd-Elhady Naiel and Khaled A. El-Tarabily, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval This literature does not contain any studies with animals performed by any of the authors.

Conflicts of interest All authors confirmed that there is no conflict of interest.

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Authors and Affiliations

Mohammed Abd-Elhady Naiel[1] · Abdelrazeq Mohamed Shehata[2,3] · Vinod Kumar Paswan[3] · Mourad Ben Said[4,5] · Khaled A. El-Tarabily[6,7]

✉ Mohammed Abd-Elhady Naiel
mohammednaiel.1984@gmail.com

✉ Khaled A. El-Tarabily
ktarabily@uaeu.ac.ae

[1] Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt

[2] Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo 11651, Egypt

[3] Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, India

[4] Laboratory of Microbiology, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, 2010 Manouba, Tunisia

[5] Department of Basic Sciences, Higher Institute of Biotechnology of Sidi Thabet, University of Manouba, 2010 Manouba, Tunisia

[6] Department of Biology, College of Science, United Arab Emirates University, 15551 Al Ain, United Arab Emirates

[7] Harry Butler Institute, Murdoch University, Murdoch, WA 6150, Australia