

IMPLICATION OF ALTERNATIVE DRUG SOURCES TO COMBAT AGAINST FISH FUNGAL PATHOGEN SAPROLEGNIA PARASITICA

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ABSTRACT

Saprolegnia parasitica is an important fish fungal pathogen, causing a significant economic challenge in aquaculture. This present review shares insights into the development of effective pharmacological components to restrict *Saprolegnia parasitica* mediated infections. Pathogenic oomycetes can infect various plant and animal hosts, leading to several diseases that significantly impact the economy. *Saprolegniasis* is a condition that impacts young fish and eggs in hatcheries around the globe. This condition is brought about by the harmful oomycete known as *Saprolegnia parasitica*. As preliminary symptoms of the disease, greyish-white marks of filamentous mycelium appear on the different parts of the fish body, including fins. Such changes promote tissue damage and even death of the organism. *Saprolegniasis* can be managed with Malachite green, but due to its harmful and cancer-causing effects on humans its application was prohibited since 2002. However, ban of Malachite green has led to enhanced occurrence of *saprolegniasis* in fish affecting the entire fisheries industry once again, thus upsetting the fishery associated global economic status. Under such circumstances, exploration of alternative ways to manage

the pathogen *Saprolegnia parasitica* seems to be relevant and crucial as well. Thus, the present review is an attempt to gather the available information on the topic keeping heed attention to discuss the probable management strategies and possible drug targets that can be brought into account to prevent Saprolegniasis.

Keywords: *Saprolegnia parasitica*, *Saprolegniasis*, *Oomycete*, *Fish Pathogen*, *Fungal Disease*, *Alternative drug sources*

1. INTRODUCTION:

Oomycetes are a diverse group of filamentous organisms that were once classified as fungi due to their structural similarities and growth patterns. However, advancements in genetic and biochemical research have reclassified them under the group Stramenopiles, which also includes diatoms and kelp [Kamoun, 2003]. The three subclasses of oomycetes, Saprolegniomycetidae, Rhipidiomycetidae, and Peronosporomycetidae, are capable of infecting a variety of hosts starting from vertebrate animals to economically significant plants [van West P, 2006]. While sexual and physical traits have historically been used to categorize the different species belonging to the genus (*Saprolegnia*), current molecular characterization using ribosomal DNA (rDNA) repeat revealed the phylogenetically divergent status of the genus. Species of *Saprolegnia* include *S. parasitica*, *S. australis*, *S. ferax*, and *S. diclina* [Molina et al., 1995]. Among these, *S. parasitica* is considered as a potential threat to the aquaculture industry. Frequently referred to as cotton mold, because of its unique characteristic white or, gray fibrous patchy appearance they form, *Saprolegnia* species are common water molds. The fish-pathogenic oomycete *Saprolegnia parasitica* is evident to cause a disease state known as saprolegniasis, that can affect fish, amphibians, and other aquatic species, leading to substantial financial losses in aquaculture that consequently declines wild fish populations and their worldwide distribution gets hampered. Saprolegniasis affects mainly incubating eggs and aquaculture broodfish where it is prevalent in the hatched salmon population. Estimated values further indicated that about 10% of the freshly hatched salmon succumb to the disease, causing significant losses to

the aquaculture industry that supplies a significant portion of fish worldwide for human consumption [Molina et al., 1995]. With over 80% of aquaculture production originating in Asia, saprolegniasis is common in tropical systems [Karunasagar et al., 2003]. In freshwater environments, *Saprolegnia parasitica* is a pervasive water mold and a major cause of fish disease. It can be found on both living and decaying organisms, often consuming saprophytes that decompose organic debris in water. *Saprolegnia parasitica* reproduces asexually through zoospores and sexually. Asexual stages are associated with saprolegniasis onset. Nutrient depletion triggers sporulation, forming sporangia at hyphal tips that release primary biflagellate zoospores, which infect host fish. These zoospores encyst into primary cysts, releasing motile secondary zoospores. Secondary cysts then produce laterally biflagellate zoospores, considered the infectious stage [Andersson and Cerenius, 2002].

Symptoms of saprolegniasis include sunken eyes, depigmented skin, hemorrhagic ulceration, cotton-like growths on the host's or eggs' skin and gills, and erosion of the skin, fins, gills, and muscles. Systemic mycosis affecting the liver, spleen, eyes, and kidney is also observed. *S. parasitica* has been evident to enter the body of adult fish through the epidermal tissues present either in the head or, in the fins and then spread throughout the entire body. However, in eggs it causes profuse mycelial development and enhances the probability of mortality of the fish [van West et al., 2010]. Possible triggers for saprolegniasis include low water temperatures, skin injuries, crowded living conditions, contamination, and bacterial or viral infections.

Treatments for saprolegniasis include potassium permanganate, copper sulfate, new methylene blue, malachite green, benzalkonium chloride, and saltwater therapies. Azelaic acid, sulfonamide sulfamethoxazole, and chloramphenicol are also effective in inhibiting *Saprolegnia parasitica* growth [Kumar et al., 2020]. Additionally, antibiotics like Cladomarine from *Penicillium coralligerum* and Quellenin from *Aspergillus* sp. exhibit anti-*Saprolegnia* properties. Notably, *Saprolegnia parasitica* does not affect humans [Kumar et al., 2020]. Application of Malachite green

was found to be beneficial in restricting the growth of *S. parasitica* in aquaculture; however, concerns regarding its toxic and carcinogenic effects led to the ban of this chemical globally since 2002[Torto-Alalibo et al., 2005]. Thus, its high time to generate useful data for the better understanding of the physiology of the parasite and its modes of pathogenesis. Moreover, knowledge on its different developmental stages and host selection criteria will help in designing an effective way to restrict the progression of saprolegniasis. Now a days, plant-based extracts comprising specific phytochemicals, as a safer and eco-friendly alternative, are suggested as beneficial options due to their potent antifungal properties. In addition, knowledge on the immunological response of the host fish will encourage to develop *S. parasitica* antibodies and may also contribute to the development of vaccines as early precautionous measure to combat and detect saprolegniasis.

2. ROUTE OF INFECTION AND PATHOGENESIS OF *Saprolegnia parasitica*:

In freshwater fish, a fungal pathogen (*Saprolegnia parasitica*) is the primary host of the water mold that causes saprolegniosis primarily in different fish species where it induces epidermal deterioration and promotes macrophage recruitment[van West et al., 2010]. *S. parasitica* has been evident to produce an eicosanoid precursor, arachidonic acid, which is known to downregulate MH II by reducing fish macrophage activity [Torto-Alalibo et al., 2005]. Usually, the characteristic gray or, cottony white marks throughout the body including fins, gills, and skin of fish confirms the infection of this virus. It is a major threat to aquaculture as well as wild fish populations, especially salmon and trout[Kales et al., 2007]. The pathogen *Saprolegnia parasitica* is known for its flexibility and wide-ranging dispersion because it can tolerate a wide range of water and temperature conditions. Reports suggested that, *S. parasitica* has been often found to act as a major pathogen, that rather being a secondary one.

Symptoms of *Saprolegnia parasitica* infections include organ failure, respiratory failure, and hemodilution. Most of the time, they can be fatal. When seen under a microscope, fish with morphology exhibit long, branching filaments known as

hyphae, as well as skin, gills, and fins coated with cotton-like white or, gray patches. It has the ability to reproduce both sexually and asexually. In asexual reproduction, movable spores called zoospores are created and released into the water. It is possible for these spores to infect new hosts. Oospores that are resistant to weather and have strong walls are produced by sexual reproduction [Torto-Alalibo et al., 2005; Kales et al., 2007; Rezinciuc et al., 2018].

Saprolegniosis is caused by pathogenicity, which primarily affects fish. The infection may cause significant mortality in fish populations, both wild and farmed. In its life cycle, the mold passes through stages where it attaches itself to the host, penetrates the tissues, and matures into sporangia that release zoospores to disperse the infection. In aquatic environments, *Saprolegnia parasitica* poses a serious threat due to its virulence. It adheres to stressed fish tissues, penetrates the skin, gills, and fins, and releasing enzymes like lipases and proteases to break down host tissues for deeper penetration and nutrient absorption. It can modulate the immune response of the host organism and thus, maintains the state of infection along with increasing disease severity [Torto-Alalibo et al., 2005; Kales et al., 2007]. This includes protection from being discovered and destroyed by the host's immune cells. It is more likely to infect more hosts as it possesses the capacity to disseminate swiftly in water because of the production of motile zoospores. Detailed ultramicroscopic studies on *S. parasitica* confirmed the presence of elongated hooked hair bundles ($10.14 \pm 1.40 \mu\text{m}$) in the cysts and cell wall that might have some implication in its pathogenesis [Rezinciuc et al., 2018]. Its resilience and spread across many aquatic environments are facilitated by its capacity to tolerate a wide range of temperatures and water conditions reproduction [Rezinciuc et al., 2018].

3. DIVERSE FUNCTIONAL PROTEINS OF *Saprolegnia parasitica*:

Sl. No.	Protein	Function	Reference
1	SpHtp1 RxLR effector protein Htp1	Binds to a tyrosine -o-fish cell surface ligand	Choudhury A, Kumar P, Nafidi HA. <i>et al.</i> (2024) Immunoinformatics approaches in developing a novel multi-epitope chimeric vaccine protective against <i>Saprolegnia parasitica</i> . Scientific Reports 14, 2260.
2	SpHtp3	Binds to the host cell	Trusch F, Loebach L, Wawra S. <i>et al.</i> (2018) Cell entry of a host-targeting protein of oomycetes requires gp96. Nature Communications 9, 2347.
3	SPRG19320 Spondin-like TSP1 domain-containing protein	It encodes a 936 amino acid protein	Jiang, R. H., de Bruijn, I., Haas, B. J., Belmonte, R., Löbach, L., Christie, J., ... & van West, P. (2013) Distinctive expansion of potential virulence genes in the genome of the oomycete fish pathogen <i>Saprolegnia parasitica</i> . PLoS Genetics, 9(6), e1003272.
4	SPRG_18636 Fibronectin type-III domain-containing protein	It encodes a 562 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete <i>Saprolegnia parasitica</i> . PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361
5	SPRG_05384 Protein-tyrosine-phosphatase	It encodes a 6547 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete <i>Saprolegnia parasitica</i> . PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361

6	SPRG_19863 GPS domain-containing protein	It encodes a 9440 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete <i>Saprolegnia parasitica</i> . PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361
7	SPRG_03974 Fibronectin type-III domain-containing protein	It encodes a 5297 amino acid protein.	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete <i>Saprolegnia parasitica</i> . PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361

4. ROLE OF FUNCTIONAL PROTEINS IN PATHOGENESIS:

SpHtp 1 is known to bind to a tyrosine-o-fish cell surface ligand [Choudhury et al., 2024]. Numerous proteins have been reported to participate in a cascade to exert the pathogenicity of the *S. parasitica*. Usually, *S. parasitica* translocate its RxLR (‘x’ encodes any amino acid)effector protein SpHtp1, a putative protein controlled by the host targeting protein 1 (Sphtp1), in the body of the host fish. Elevated expression of this SpHtp1protein has been noted prior to the initiation of the infection and during the initial stages of pathogenesis [Epstein and Nicholson, 1997]. Similarly, SpHtp 3 is evident to bind the host cell [Trusch et al., 2018], whereas SPRG19320 helps in the formation of the secretome [Jiang et al., 2013]. Secretome formation is an important stage for pathogenesis caused by *S. parasitica*,where five functional proteins have been evidenced to be upregulated relatively higher in the cysts in comparison to the mycelia. These five proteins viz. SPRG_18636, SPRG_05384, SPRG_19863, SPRG_03974 and SPRG_19320 are known to encode 562, 6547, 9440, 5297 and 936 amino acid containing proteins, respectively. In case of the first four proteins, the suggested catalytic domain possesses one or multiple domain(s) of ScanProcite fibronectin III;

while, SPRG_19320 consists of thrombospondin domain. However, investigation applying NetNGlyc tool suggested the presence of multiple (4-20) sites for N-glycosylation in all the five putative proteins [Rezinciuc et al., 2018]. On the other hand, further studies on the host-parasite interaction revealed that specific molecules released from the oomycetes helps *S. parasitica* to attach to their host surface [Hardham, 2001; Mendgen et al., 1996; Epstein and Nicholson, 1997]. In such regards, presence of bundles of elongated hooked hair-like structures in the cysts also facilitate the pathogen to attach the fish body [Rezinciuc et al., 2018]. Despite of the fact that numerous attempts have been made to explore the mechanism of attachment of the pathogen to the host body, but the specific functions of these specialized structures remained unexplained. Such information is of eminent importance as it may help the researchers to develop a therapeutic agent to inhibit the attachment of the parasite to the host, thus, may restrict its pathogenesis in the aquatic organisms. In such regards, plant extract may serve as a natural remedial measure with less or, no toxicity in the host organism.

5. PLANT EXTRACTS AS ALTERNATIVE DRUG SOURCES:

Sl. No.	Name	Scientific name	Parts used	Reference
1	Denaee thyme	<i>Thymus daenensis</i> Celak	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

2	Khuzestani thyme	<i>Thymus khuzestanicum</i>	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
3	Mint	<i>Mentha longifolia</i> (L.) <i>Hudson</i>	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
4	Common myrtle	<i>Myrtus communis</i> L.	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

5	Tansy	<i>Tanacetum parthenium</i> (L.) Schultz-Bip	Flower	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
6	Bakhtyari savory	<i>Satureja bachtiarica</i> Bunge	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
7	Hyssop	<i>Hyssopus officinalis</i> L.	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on <i>Saprolegnia parasitica</i> from cutaneous lesions of rainbow trout (<i>Oncorhynchus mykiss</i>) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

8	Galanga	<i>Alpinia galanga</i>	Rhizome	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, <i>Saprolegnia parasitica</i> H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
9	Betel Vine	<i>Piper betel</i> Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, <i>Saprolegnia parasitica</i> H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
10	-	<i>Rhinacanthus nasutus</i> Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, <i>Saprolegnia parasitica</i> H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.

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11	-	<i>Kaempferia galanga</i> Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, <i>Saprolegnia parasitica</i> H2, from fish. Kasetart Journal (Natural Science), 41, 56-61.
12	-	<i>Kaempferia galanga</i> Linn	Root	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, <i>Saprolegnia parasitica</i> H2, from fish. Kasetart Journal (Natural Science), 41, 56-61.
13	Black caraway	<i>Nigella sativa</i>	Seeds	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against <i>Saprolegnia diclina</i> , the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.

14	Thyme	<i>Thymus vulgaris</i>	Leaves	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against <i>Saprolegnia diclina</i> , the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.
15	Pomegranate	<i>Punica granatum</i>	Peels	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against <i>Saprolegnia diclina</i> , the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.
16	Ginger	<i>Zingiber officinale</i>	Rhizome	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against <i>Saprolegnia diclina</i> , the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.

Apart from these information, several other plant extracts with established antifungal properties have been reported [Emara et al., 2020] such as- (i) Terpenoids present in plants such as eucalyptus (*Eucalyptus globulus*), tea tree (*Melaleuca alternifolia*), and neem (*Azadirachta indica*); (ii) Flavonoids present in plants such as turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and garlic (*Allium sativum*); (iii) Alkaloids present in plants such as nightshade (*Solanum* spp.) and papaya (*Carica papaya*); and (iv) Phenolic compounds present in thymesuch as (*Thymus vulgaris*), cloves (*Syzygium*

aromaticum), and cinnamon (*Cinnamomum verum*). Surprisingly, their mode of action against Saprolegniasis and the underlying molecular mechanism are yet to be explored and requires detailed research.

6. CONCLUSION:

As an offshoot of the international ban of Malachite Green in 2002, the need to develop alternative methods for the control of saprolegniasis has arisen with greater urgency. Genomic and proteomic studies of *S. parasitica* and other pathogenic oomycetes provided some good resources regarding the molecular underpinnings of saprolegniasis. Control of saprolegniasis is essential for continued advancement in the aquaculture industry, particularly in Asian tropical aquaculture systems, where more than 70-80% of fish produced by aquaculture originates from these regions. For an industry responsible for about 30% of fish consumed worldwide, the molecular processes underlying saprolegniasis in *S. parasitica*-host fish interactions must be investigated thoroughly. In such regards, discovery of plant extracts, antifungal agents, biocontrol methods, and genomic strategies should be promoted to develop effective treatments against saprolegniasis. Identification of plant extracts with strong antifungal activity against *Saprolegnia parasitica* will lead to the development of an eco-friendly, non-toxic treatment for saprolegniosis. Good environmental management practices will help reduce the pathogen's prevalence. A step toward sustainable aquaculture practices by reducing reliance on synthetic antifungal agents. It will lead to the promotion of biodiversity by using natural plant resources. Reduction in environmental and health risks associated with conventional treatments. Furthermore, ongoing research and collaboration between researchers, aquaculture professionals, and regulatory bodies are essential for the development and implementation of effective control strategies. Through research, the goal is to create sustainable and safe solutions that protect fish health and enhance aquaculture productivity, ultimately benefiting both the industry and the ecosystems involved. Exploring the combination of plant extracts with other biocontrol agents may enhance the overall efficacy

of treatments. The advancement of genomic tools and bioinformatics will aid in identifying more targeted, efficient, and sustainable treatment options. The collaboration between researchers and aquaculture professionals will be necessary for complete eradication of the pathogen from the ecosystem.

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