

PHYTOCHEMICAL PROFILING AND IN VITRO ANTIFUNGAL ACTIVITY OF EUCALYPTUS GLOBULUS LEAF EXTRACTS AGAINST FISH PATHOGENIC FUNGI

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ABSTRACT

The present investigation was carried out to evaluate the in-vitro antifungal potential of Eucalyptus globulus leaf extract against fish associated fungal isolates i.e. *Aspergillus flavus* (PP980959, PP980962), *Aspergillus fumigatus* (PP956955, PP980961), *Alternaria alternata* (PP980717), *Aspergillus* spp. refer to (PP980956) and *Alternaria* spp. (PP980711) from different aquaculture systems of Hisar district, Haryana; which were identified by using morphological, microscopic and molecular methods. E. globulus leaf extracts of aqueous, hydroethanol and absolute ethanol consists of phytochemical viz. tannins, saponins, alkaloids, glycosides, steroids, terpenoids and anthraquinones with variable intensity among the solvents. Among all the extracts, hydroethanol extract had the highest total phenolic content (86.35 ± 2.21 mg GAE/g) and total flavonoid content (24.68 ± 0.92 mg QE/g). Subsequently, absolute ethanol and aqueous next. According to FTIR result, the presence of functional groups like phenols, alcohols, esters, ethers, carbonyl compounds, and aromatic functional groups indicates the antifungal activity. The antifungal test results revealed that the inhibition was found to be concentration-dependent as 8% and 16% extracts exhibited stronger activity as compared to 2% and 4%. The aqueous extract showed an antifungal activity with zone of inhibition values from 7-15 mm which is lower than other extracts (absolute ethanol and hydroethanol). The MIC values were lowest for absolute ethanol extract at 30-75 mg/ml, followed hydroethanol (40-90 mg/ml) and aqueous extract (45-115 mg/ml). As a whole, the finding of the study reveals that Eucalyptus globulus extract, especially absolute ethanol extract, demonstrated better antifungal activity against fish-associated fungal isolates, which may be an antifungal agent for aquaculture health management after in vivo assessment.

KEYWORDS: Eucalyptus globulus, antifungal activity, zone of inhibition, Minimum inhibitory concentration

INTRODUCTION

The food production sector that is growing most rapidly in the world is aquaculture. It can make a significant contribution to food security, nutrition, livelihood support and rural economic development and rising need for fish protein has led to the growth and intensification of aquaculture systems, which includes ponds, tanks, cages and recirculatory systems. Intensive culture techniques can lead to the fish becoming stressed due to high stocking density, water quality, organic load, handling stress and quick disease transmission. Under such conditions, the immune system of cultured fish becomes weakened and more susceptible to infection. The sustainable aquaculture production of food fish has various constraints. Among the various constraints, the bacterial, viral, parasitic and fungal pathogens are amongst the important disease-causing agents [11, 21, 4, 17].

Fungal diseases are primarily considered as an important health problem in freshwater aquaculture, especially in stressed, wounded or immunosuppressive fish and become a major limitation in freshwater aquaculture, causing skin lesions, gill damage, poor growth, mortality and economic losses in cultured fish. Fungi and fungal-like organisms are present naturally in aquatic environments and generally act as opportunistic pathogens which invade damaged skin, fins, gills and eggs. Saprolegniasis is one of the common fungal-like infections caused by *Saprolegnia* spp. that have cotton-like white or greyish growth on the body surface, gills and also on the eggs [29, 28, 17]. Various true fungi including those of *Aspergillus*, *Alternaria*, *Fusarium* and *Rhizopus* species have also been reported from diseased fish and aquaculture environment. oomycetes (*Saprolegnia*, *Achlya* and *Aphanomyces*) [21, 7, 4]. These infections may cause lesions on the skin, damage to gills, lower growth, poor sale ability, secondary infections and mortality. Traditionally, the fungal diseases in fish culture management were controlled by chemical agents like malachite green, formalin, copper sulphate, potassium permanganate, hydrogen peroxide and salt. Even though malachite green was regarded as highly effective against fungal infections and external parasites used in food fish, it has been banned or restricted in many countries due to the toxic, mutagenic, teratogenic and carcinogenic effects [24, 25, 17]. Excessive use of synthetic substances can lead to build-up,

environmental contamination, non-target organism toxicity and consumer safety concerns. As a result, the world needs safer, eco-friendly, biodegradable and cost-effective alternatives for the management of fungal diseases in aquaculture [23,16].

Over the years, medicinal plants have gained importance as natural alternatives to antibiotics and synthetic chemicals for aquaculture health management. Plant extracts, which possess bioactive compounds like phenols, flavonoids, tannins, alkaloids, terpenoids, saponins, quinones and essential oils, may exhibit antimicrobials, antifungals, antioxidants and anti-inflammatory and immunostimulatory properties [16, 23]. *Eucalyptus globulus* is a plant with anti-microbial and anti-fungal potential. The leaves of this plant are reported to contain primary metabolites, secondary metabolites and essential oil which is rich in 1,8-cineole/eucalyptol. In addition, the compound also consists of other phenolic and terpenoid compounds that can disrupt cell walls and membranes of fungi. Antifungal activity is also influenced by the extraction solvent as ethanol and hydroethanol may extract more active phytochemicals than water [23]. Though many studies have reported antimicrobial efficacy of its essential oils and extracts, not much information is available on the solvent-dependent antifungal efficacy of its leaf extract against fungal isolates from fish culture systems. Hence, the present study was conducted to evaluate *in vitro* antifungal activity of *Eucalyptus* extract against fungal isolates associated fish. The objective was to compare the extracts prepared in distilled water, hydroethanol and absolute ethanol and the efficacy was measured for inhibitory activity and minimum inhibitory concentration was determined. This suggests *E. globulus* has potential as a natural antifungal agent; however, further studies for *in vivo* toxicity, safety and field level validation are required before application at the fish farm level.

MATERIALS AND METHODS

(a) Fungal Strains: The fungal strains used in the present study were obtained from diseased fish and aquaculture water samples collected from different fish culture systems. The isolates were characterized based on colony morphology, microscopic features and molecular identification using ITS sequencing. The selected fungal isolates included *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Aspergillus* sp. and *Alternaria* sp. These isolates were maintained on Sabouraud Dextrose Agar (SDA) plates and sub-cultured before use in the antifungal assay. Fresh fungal cultures were used for the preparation of inoculum to evaluate the antifungal efficacy of *E. globulus* leaf extract.

(b) Preparation of extract of *Eucalyptus globulus* globus leaves: The *E. globulus* leaves extract was prepared in three different solvents i.e., distilled water, Hydroethanol and absolute ethanol. The leaves were collected from the local forest of university campus. The confirmation of the identified species was done with the help of Department of Botany, CCS HAU, Hisar. Collected leaves were washed with Aqueous and then chopped. Leaves were dried in the shade and then grinded. A preliminary study was conducted to finalize the best composition of solvent to extract the maximum number of phytochemicals like phenol, alkaloid, ascorbic acid, flavonoid, saponin, tannin and other antioxidants from the leaves and for the preparation of 10% (w/v) extract of its leaves, 10 gms of grounded samples were soaked in 100 ml of 90% alcohol/distilled water/absolute ethanol and kept in the shaker for 48 h. Mixture was filtered through Whatman filter (No.1) paper and then centrifuged at 10000 g for 10 min. Supernatant was collected and kept in an amber-coloured bottle at 4 °C until further use. The supernatant was then evaporated in the rotary evaporator at 40°C. The powder obtained from rotatory evaporator was further used to prepare solutions of 2%, 4%, 8%, 16% w/v. [30, 31].

(c) 2.3 Preliminary Phytochemical Screening: Preliminary phytochemical screening of *E. globulus* leaf extract was carried out to detect the presence of major secondary metabolites such as phenols, flavonoids, tannins, alkaloids, saponins, terpenoids and other bioactive compounds. Standard qualitative tests were performed following established phytochemical screening procedures described for medicinal plant extracts [27]. The presence or absence of different phytoconstituents was recorded based on characteristic colour change, precipitation, froth formation or ring formation depending on the specific test. Similar phytochemical screening of *E. globulus* leaf extract has reported the presence of alkaloids, flavonoids, phenols, tannins, quinones, glycosides, steroids and terpenoids, which supports its antimicrobial and antifungal potential. Phytochemical screening was considered important because plant secondary metabolites, particularly phenolic compounds, flavonoids and terpenoids, are commonly associated with antimicrobial activity through disruption of microbial cell walls, alteration of membrane permeability, enzyme inhibition and leakage of intracellular contents [2; 22].

(d) Estimation of Total Phenolic and Flavonoid Content: The total phenolic content of *Eucalyptus globulus* extract was estimated using the Folin-Ciocalteu reagent method, in which the plant extract was reacted with Folin-Ciocalteu reagent and sodium carbonate, followed by incubation in dark conditions and spectrophotometric absorbance measurement. The phenolic content was expressed as gallic acid equivalent using a gallic acid standard curve [3]. Similarly, total flavonoid content was determined using the aluminum chloride colorimetric method, where the extract was mixed with aluminum chloride reagent and absorbance was measured after incubation. The flavonoid content was expressed as quercetin equivalent using a quercetin standard curve [8]. These estimations were useful for relating the antifungal activity of *E. globulus* leaf extract with the presence of phenolic and flavonoid compounds, as these phytochemicals are known to contribute to antimicrobial and antifungal potential in plant extracts.

(e) FTIR Analysis of Prepared *Eucalyptus globulus* Extract: Fourier Transform Infrared (FTIR) spectroscopy was used to determine the functional groups and chemical bonds of the *E. globulus* leaf extract. FTIR is a reliable analytical technique for activity tracking due to its ability to generate a molecular fingerprint of the extracts based on the absorption peaks created by the vibration of the chemical bonds of bioactive compounds [14, 5]. FTIR analysis of *E. globulus* extract detects the presence of functional groups associated with phenols, flavonoids, tannins, alcohols, esters, amines, aromatic compounds and constituents of essential oils, which may confer antimicrobial and antifungal activity [5,15]. The infrared radiation was directed to the extract. The absorption peaks corresponding to various wavenumbers were then recorded to reveal the specific molecular vibrations. The FTIR spectrum that was obtained was analyzed with the aid of program to

comprehend the chemical nature. FTIR analysis of Eucalyptus globulus extract prepared in various solvents (aqueous, absolute ethanol, hydroethanol) was carried out Central Laboratory, CCSHAU, Hisar in the present study.

(f) In Vitro Antifungal Assay: The in vitro antifungal activity of E. globulus extract was evaluated using the agar well diffusion method [6]. Sabouraud Dextrose agar medium was prepared, sterilized and poured into sterile petri plates and after solidification, fungal inoculum was evenly spread on the agar surface using a sterile cotton swab. Wells were made in the agar plates using a sterile borer, and different concentrations of E. globulus leaves extract were added into the wells [13]. Plates were incubated under suitable conditions, and fungal growth inhibition was observed after incubation. Comparison of extracts prepared in distilled water, hydroethanol and absolute ethanol was made for determining the effect of solvent. Inhibition should be confirmed by using solvent control so that it can be said to be due to plant extract.

RESULT AND DISCUSSION

(i) Identification of Fungal strains

Fungal isolates from infected fish samples obtained from different farms located in Hisar district, Haryana by purification and repeated sub-culturing on the fungal growth media then characterized based on their respective colony morphology and microscopic features. The selected filamentous fungal isolates were molecularly identified using ITS sequencing. The team sequenced the purified PCR products and analysed these sequences by BLASTn for species confirmation. Following validation of the sequence, the identified fungal isolates were submitted to a National Center for Biotechnology Information database, and accession numbers were obtained. The table reveals the fungal strains utilized in the in vitro antifungal activity assay of the extract of Eucalyptus globulus.

Table 1. Fungal strains used in the present study

S. No.	Fungal species	Source of isolate	Strain code	NCBI Accession No.
1	Aspergillus flavus	Body surface of Clarias batrachus	HAUAAHM_AF1	PP980959
2	Aspergillus fumigatus	Gills of Channa striata	SS1	PP956955
3	Alternaria alternata	Body surface of Labeo rohita	HAUAAHM_AA1	PP980717
4	Aspergillus flavus	Body surface of Pangasius hypophthalmus	HAUAAHM_AF2	PP980962
5	Aspergillus fumigatus	Body surface of Channa striata	HAUAAHM_AF2	PP980961
6	Aspergillus sp.	Body surface of Pangasius hypophthalmus	HAUAAHM_A1	PP980956
7	Alternaria sp.	Body surface of Labeo rohita	HAUAAHM_A1	PP980711

(ii) Preliminary Phytochemical Screening

Preliminary phytochemical screening of E. globulus leaf extract showed the presence of different secondary metabolites in extracts prepared using distilled water, hydroethanol and absolute ethanol. The intensity of phytochemical reaction varied with the extraction solvent, indicating that solvent polarity influenced the extraction of bioactive compounds. Tannins were strongly present in all extracts, while saponins were detected in distilled water, hydroethanol and absolute ethanol extracts with variable intensity. Alkaloids were observed only in the Aqueous extract, whereas glycosides, steroids and anthraquinones showed stronger reactions in the absolute ethanol extract. The hydroethanolic extract showed a comparatively broad phytochemical profile, which may be due to the combined extraction efficiency of water and ethanol for both polar and moderately polar compounds. Previous studies have also reported that E. globulus leaves contain phenolic metabolites, flavonoids and other bioactive compounds, and that ethanol concentration influences the recovery of these phytochemicals [18, 22].

Table 2. Preliminary phytochemical screening of E. globulus leaf extracts prepared in different solvents

S. No.	Phytochemical	Aqueous extract	Hydroethanol extract	Absolute ethanol extract
1	Tannins	+++	+++	+++
2	Saponins	++	++	+
3	Alkaloids	+	-	-
4	Flavonoids	-	+	-
5	Glycosides	-	++	++
6	Steroids	+	++	+++
7	Terpenoids	-	+	-
8	Anthraquinones	-	++	+

Note: “+++” = strongly present, “++” = moderately present, “+” = weakly present, and “-” = absent.

(iii) Total phenolic and flavonoid content

The total phenolic and flavonoid content of *E. globulus* leaf extracts varied with the solvent used, with hydroethanol extract showing the highest values (86.35 ± 2.21 mg GAE/g and 24.68 ± 0.92 mg QE/g), followed by absolute ethanol and aqueous extracts, indicating that hydroethanol is more effective due to its intermediate polarity [22, 18]. Absolute ethanol showed moderate phenolic content but lower flavonoids, while aqueous extract had the lowest values, suggesting water alone is less efficient for extracting these compounds [3]. The higher phenolic and flavonoid content in hydroethanol extract may enhance its biological activity, as these compounds possess antioxidant and antifungal properties by disrupting fungal cell structures and metabolism [23], although higher antifungal activity in ethanol extract, if observed, may be due to other bioactive compounds such as terpenoids and essential oils.

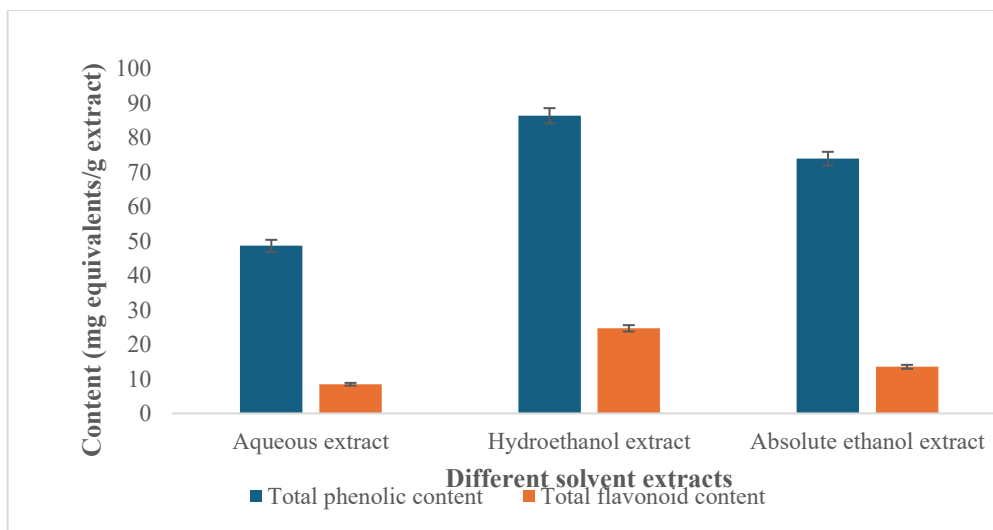


Fig. 1: Total phenolic and flavonoid content of *Eucalyptus globulus* leaf extracts prepared using different solvents.

(iv) FTIR Analysis of *Eucalyptus globulus* Extract Prepared in Aqueous Solvent

The FTIR spectrum of *E. globulus* extract prepared in aqueous solvent is shown in Fig. 22 and revealed several absorption peaks indicating the presence of different bioactive functional groups. The broad peak at 3341.12 cm^{-1} represents O–H stretching of alcohols and phenolic compounds, and may also indicate N–H stretching of amines or amides, suggesting the presence of polar phytochemicals. The peaks at 1628.70 cm^{-1} and 1517.18 cm^{-1} indicate C=C stretching of aromatic or alkene compounds and possible N–H bending vibrations, while the peak at 1449.26 cm^{-1} corresponds to CH_2/CH_3 bending vibrations. The absorption bands at 1243.01 cm^{-1} and 1054.85 cm^{-1} are mainly associated with C–O stretching of alcohols, ethers and esters, or C–N stretching of amines, indicating the presence of oxygenated compounds commonly found in plant extracts. The lower-frequency peaks at 550.30 , 456.01 and 421.86 cm^{-1} fall in the fingerprint region and may be due to skeletal or bending vibrations of complex organic molecules. Overall, the FTIR profile confirmed the presence of phenols, alcohols, esters, ethers, amines, amides and aromatic compounds in the aqueous *E. globulus* extract, which may contribute to its antifungal potential against fish-associated fungal isolates [5]; [15].

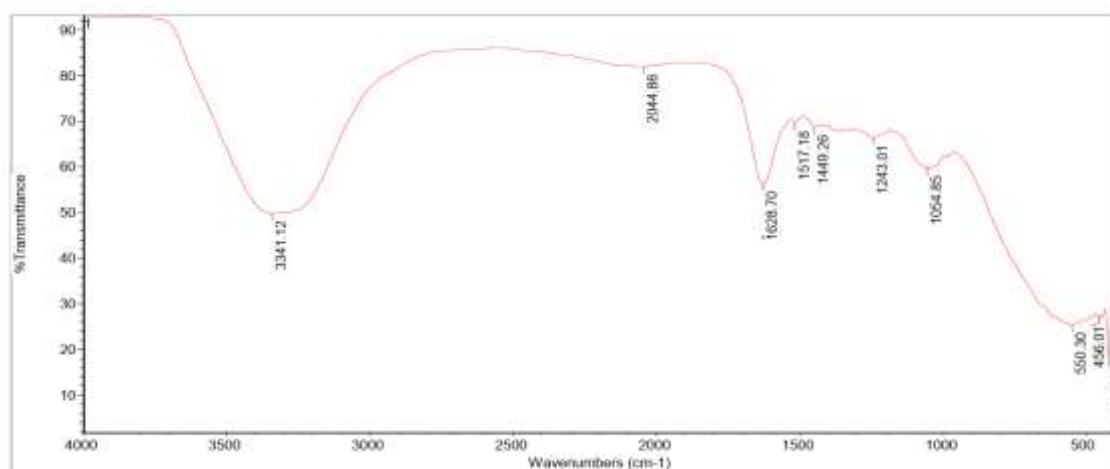


Fig. 2: FTIR spectrum of *E. globulus* extract prepared in aqueous Solvent

(iv) FTIR analysis of *E. globulus* extract prepared in hydro-ethanol

The FTIR spectrum of *E. globulus* extract prepared in hydro-ethanol, as shown in Fig. 3, confirmed the presence of several bioactive functional groups. The broad peak at 3347.46 cm^{-1} indicated O–H stretching of alcohols and phenolic

compounds, while peaks at 2973.37, 2926.47 and 2898.21 cm^{-1} represented C–H stretching of alkanes. The absorption peaks at 1684.76 and 1639.31 cm^{-1} were associated with C=O stretching and C=C stretching of aromatic or alkene compounds. Peaks at 1450.51, 1378.92, 1272.37, 1084.66 and 1043.33 cm^{-1} indicated CH_2/CH_3 bending and C–O stretching of alcohols, ethers, esters and phenolic compounds. Overall, the FTIR profile suggests the presence of alcohols, phenols, esters, ethers, carbonyl compounds and aromatic groups in the hydro-ethanol extract, which may contribute to the antifungal potential of *Eucalyptus globulus* against fungal isolates [5].

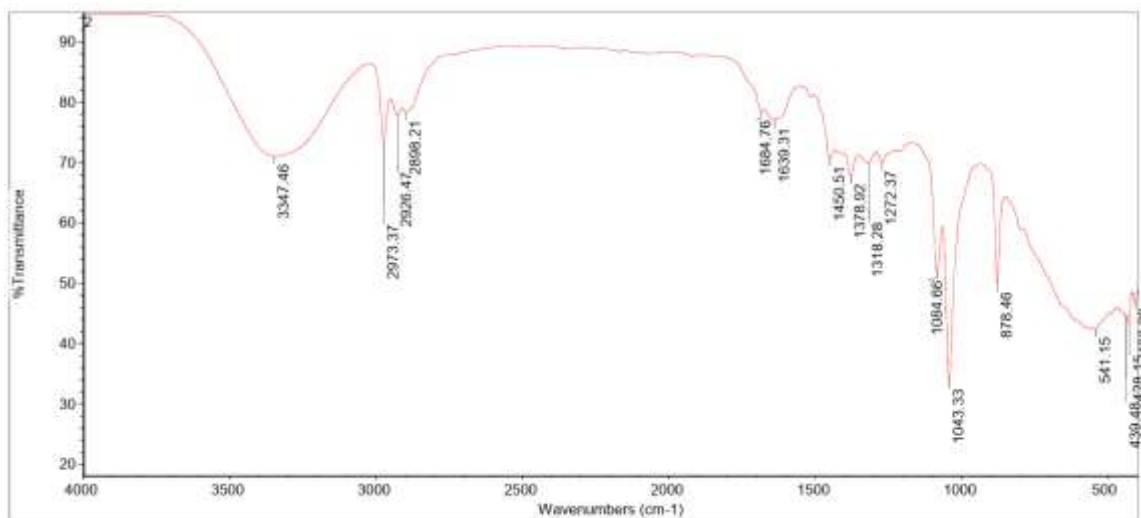


Fig. 3: FTIR spectrum of *Eucalyptus globulus* extract prepared in hydro-ethanol

3 FTIR analysis of *Eucalyptus globulus* extract prepared in absolute ethanol

The FTIR spectrum of *Eucalyptus globulus* extract prepared in absolute ethanol showed in figure important absorption peaks confirming the presence of bioactive functional groups. The broad peak at 3383.82 cm^{-1} indicated O–H stretching of alcohols and phenolic compounds, while peaks at 2925.48 and 2856.30 cm^{-1} represented C–H stretching of aliphatic compounds. Peaks at 1686.84, 1644.63 and 1512.93 cm^{-1} suggested C=O stretching, C=C stretching and aromatic ring vibrations, indicating carbonyl and aromatic phytochemicals [5]. The bands at 1452.98, 1376.94, 1252.43, 1169.51, 1086.32 and 1043.69 cm^{-1} corresponded to CH_2/CH_3 bending and C–O stretching of alcohols, ethers, esters and phenolic compounds [15]. Overall, the spectrum confirmed the presence of phenols, alcohols, esters, ethers, carbonyl compounds and aromatic groups, which may contribute to the antifungal activity of *E. globulus* extract.

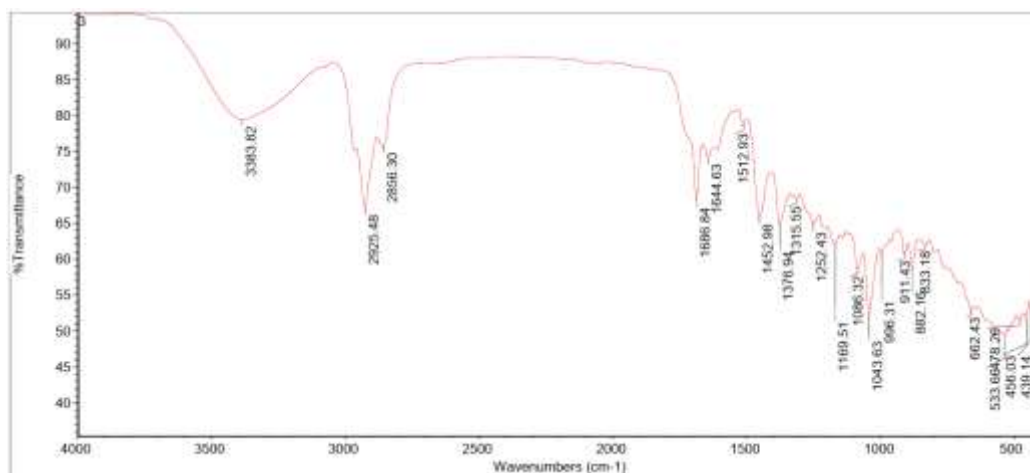


Fig. 4: FTIR spectrum of *E. globulus* extract prepared in ethanol

(v) Antifungal Activity of *E. globulus* Extract at Various Concentrations

Table 3: antifungal activity of *E. globulus* extracts

S.N o.	Fungal sp.	Accession no.	Absolute ethanol (mg/ml)				Hydroethanol(70%) (mg/ml)				Aqueous(mg/ml)			
			2%	4%	8%	16%	2%	4 %	8%	16 %	2%	4%	8%	16 %
1.	<i>Aspergillus flavus</i>	PP980959	-	+	+	+	-	-	+	+	-	-	+	+

2.	Aspergillus fumigatus	PP956955	-	-	+	+	-	-	-	+	-	-	-	+
3.	Alternaria alternata	PP980717	-	-	+	+	-	-	+	+	-	-	-	+
4.	Aspergillus flavus	PP980962	-	+	+	+	-	-	+	+	-	-	+	+
5.	Aspergillus fumigatus	PP980961	-	-	+	+	-	-	-	+	-	-	-	+
6.	Aspergillus spp.	PP980956	-	+	+	+	-	-	+	+	-	-	-	+
7.	Alternaria spp.	PP980711	-	-	+	+	-	-	+	+	-	-	-	+

Table 3 shows that the antifungal activity of *E. globulus* extract increased with increasing concentration, where 8% and 16% extracts showed better inhibition than 2% and 4% against the tested fungal isolates. Absolute ethanol extract showed comparatively stronger activity, as inhibition was observed at lower concentrations against *Aspergillus flavus* and *Aspergillus* spp., while hydroethanol and Aqueous extracts generally required higher concentrations to produce inhibition. This pattern suggests that ethanol-based solvents may extract more antifungal phytochemicals such as phenols, terpenoids and essential oil constituents, which are known to affect fungal growth by damaging cell membranes and interfering with metabolic activity [9, 18]. Similar concentration-dependent antifungal activity of *E. globulus* extracts or oils has been reported against fungal pathogens including *Aspergillus* spp. and other filamentous fungi [2, 10, 26]. Essential oils and plant-derived bioactive compounds have also been reported to show inhibitory effects against *Aspergillus flavus*, *A. fumigatus* and related fungi, supporting the antifungal potential of herbal extracts [1]. In aquaculture, plant extracts are increasingly studied as safer alternatives for fungal disease control because chemical antifungals have toxicity and residue-related limitations [19, 17]. Overall, the results indicate that the antifungal effect of *Eucalyptus globulus* extract was concentration- and solvent-dependent, with absolute ethanol being the most effective solvent, followed by hydroethanol and distilled water.

(vi) Minimum inhibitory concentration of *Eucalyptus globulus* extract against different fungal isolates

As shown in the graph, the MIC values of *E. globulus* extract varied among the tested fungal isolates and extraction solvents. Lower MIC values indicate stronger antifungal activity [6], and this pattern was clearly observed for absolute ethanol extract, which showed the lowest MIC values against all isolates. For *Aspergillus flavus*, MIC values were 30, 40 and 45 mg/ml for absolute ethanol, hydroethanol and distilled water, respectively, while another *A. flavus* isolate showed 35, 55 and 70 mg/ml. Similarly, *Aspergillus fumigatus* showed higher MIC values of 75, 90 and 115 mg/ml, and another isolate showed 60, 85 and 95 mg/ml, indicating comparatively lower sensitivity. *Alternaria alternata* showed MIC values of 50, 70 and 80 mg/ml, whereas *Aspergillus* spp. and *Alternaria* spp. showed 35, 50, 65 mg/ml and 60, 75, 90 mg/ml, respectively. The absolute ethanol extract was most effective, followed by hydroethanol and distilled water. This solvent-dependent antifungal activity may be due to better extraction of phenols, terpenoids and essential oil components in ethanol-based solvents [9], which are known to damage fungal cell membranes and inhibit fungal growth [2, 26].

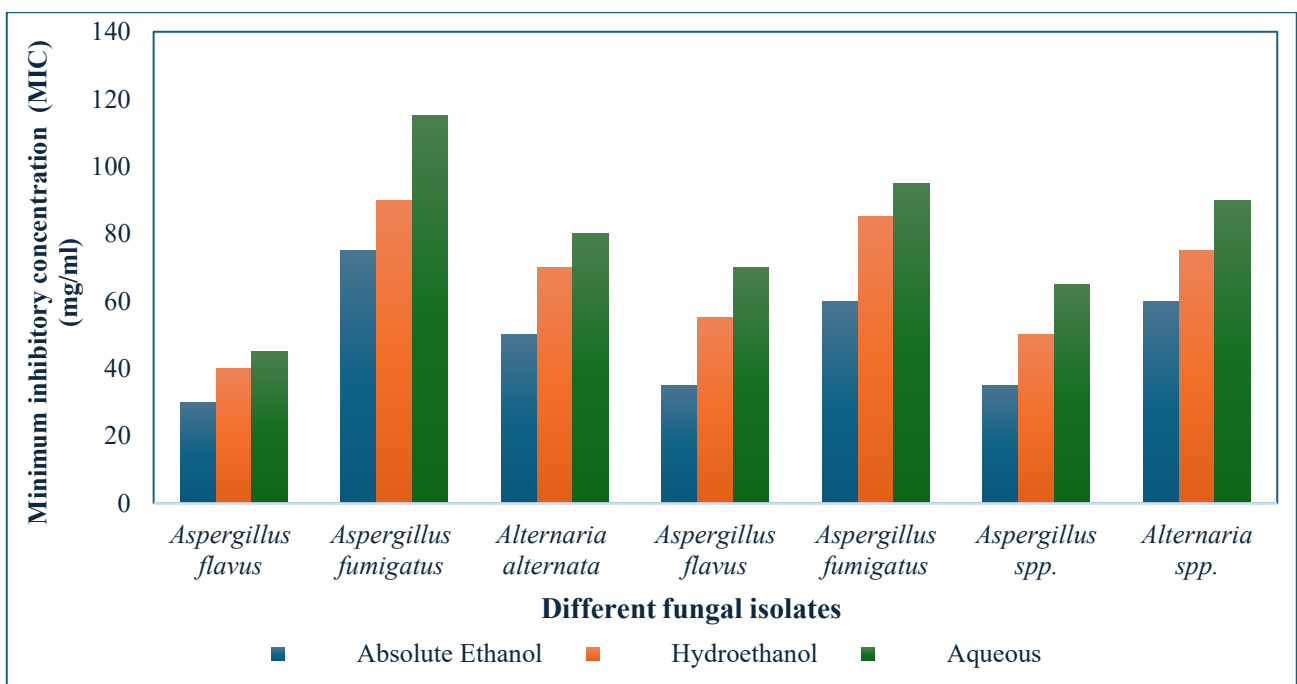


Figure 5: Zone of Inhibition of *Eucalyptus globulus* Extract Against Different Fungal Isolates

As shown in the graph, *Eucalyptus globulus* extract at 8% w/v produced clear zones of inhibition against all tested fungal isolates, but the inhibition varied according to solvent type and fungal species. Absolute ethanol extract showed the highest inhibition zone, ranging from 16–25 mm, followed by hydroethanol extract with 12–21 mm and Aqueousextract with 7–15 mm. The highest inhibition was recorded against *Alternaria alternata* with absolute ethanol extract (25 mm), followed by *Aspergillus fumigatus* PP980961 (23 mm), while the lowest inhibition was observed in Aqueousextract against *Aspergillus fumigatus* PP956955 (7 mm). This result indicates that ethanol-based extracts were more effective than aqueous extract, probably due to better extraction of phenolic compounds, terpenoids and essential oil constituents, which are known to affect fungal cell membrane integrity and inhibit fungal growth [9, 20]. Similar antifungal activity of *Eucalyptus globulus* leaf extracts against fungal isolates such as *Aspergillus flavus* and *Aspergillus fumigatus* was reported by [2, 22, 10] also reported strong antifungal activity of *Eucalyptus globulus* essential oils against filamentous fungi. Further, [26] and [1] supported the concentration-dependent antifungal potential of *Eucalyptus globulus* extracts and essential oils. Overall, the graph confirms that absolute ethanol was the most effective solvent, followed by hydroethanol and distilled water.

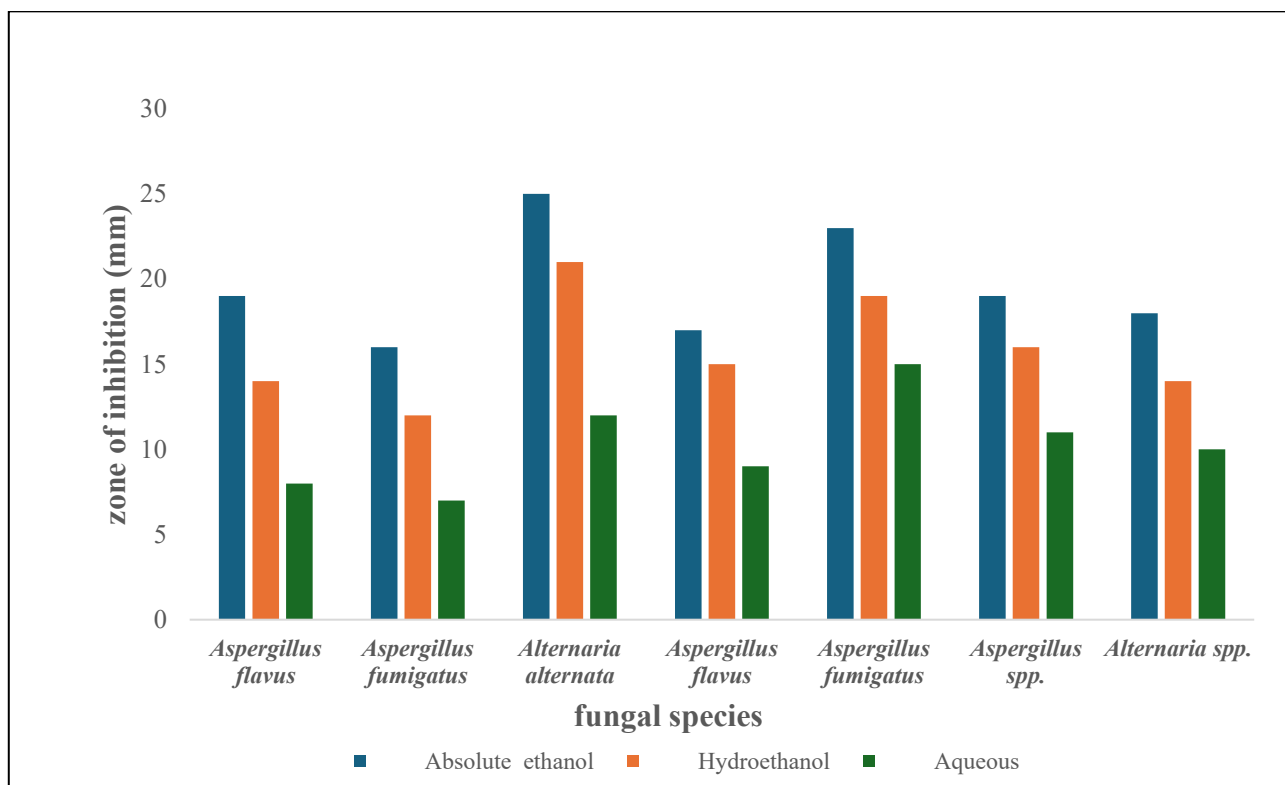


Figure 6: Comparison of Antifungal Activity of Different Solvent Extracts Against Various Fungal Species

CONCLUSION

The present study demonstrated that *E. globulus* leaf extract possesses promising in vitro antifungal activity against fish-associated fungal isolates recovered from different aquaculture systems of Hisar district. The selected fungal isolates, including *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Aspergillus spp.* and *Alternaria spp.*, were molecularly confirmed and used for antifungal evaluation. Preliminary phytochemical screening, total phenolic and flavonoid estimation, and FTIR analysis confirmed the presence of important bioactive compounds such as phenols, flavonoids, tannins, alcohols, esters, ethers, carbonyl compounds and aromatic groups, which may be responsible for antifungal activity. The antifungal response was concentration- and solvent-dependent, with stronger inhibition observed at 8% and 16% concentrations. Among the solvents, absolute ethanol extract showed the highest antifungal efficacy, with the lowest MIC values and maximum zones of inhibition, followed by hydroethanol and aqueous extract. Overall, the findings suggest that *E. globulus* extract, especially absolute ethanol extract, may be considered a potential natural antifungal agent for aquaculture health management; however, further in vivo toxicity, safety and field-level validation studies are required before practical application.

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