ANTIBACTERIAL ATTRIBUTES OF VARIOUS MUSHROOM MYCELIUM EXTRACTS AGAINST ANTI –INFECTION SAFE *Aeromonas hydrophila*

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Abstract

The present study manages the antibacterial movement of the different mushroom extricate against *A. hydrophila*. The natural product assortments of mushrooms were gathered and washed. At that point exposed to tissue culture. Mycelium was removed utilizing various solvents. The antibacterial investigations demonstrated critical inhibitory movement against the tried bacterium of *A. hydrophila.* The restraint zones delivered were fundamentally ($p<0.05$) higher for the ethyl acetic acid derivation concentrate of *C. indica, Tricholoma sp1* and *G.lucidium* when contrasted with the positive control of chloramphenicol and streptomycin. Among the over three mushroom concentrate of *C. indica* demonstrated the most extreme zone of hindrance (16 ± 0.57mm) against invitro development of *A. hydrophila* when contrasted with the positive control chloramphenicol (30 ± 0.57mm). Among the antibacterial movement of anti-toxins a greatest zone of restraint was seen in chloramphenical (30 ± 0.57mm) and least was seen in pencilin (3 ± 0.57mm). The MIC of tried mixes was gone from 20µl/ml to 80µl/ml. Among contagious tried, *C. indica* and *Tricholoma sp1* demonstrated the most noteworthy movement (40µl/ml and 60µl/m) against *A. hydrophila*. The most minimal inhibitory movement was seen in oil ether concentrate of *L. squarrosulus* (80µl/ml). As per the consequences of MIC, the ethyl acetic acid derivation concentrate of *C. indica* may be utilized as antibacterial specialist against *A. hydrophila*. From the DNA fracture investigation, the ethyl acetic acid derivation concentrate of *C. indica* (40µl/ml) blended bacterial suspension demonstrated the corroborative outcome for DNA fracture, when contrasted and the control (live *A. hydrophila*). Extremely clear divided DNA band was seen in the tried path 2 (separate blended in with *A. hydrophila*) and path 3 (streptomycin with *A. hydrophila*). Discontinuity was not seen in path 1

(*A. hydrophila* alone). This outcome showed that the ethyl acetic acid derivation concentrate of *C. indica* can lessen or control the development of bacterial states.

Key words: Mycelium extracts, *Aeromonas hydrophila*, DNA fragmentation assay, Antibiotic.

INTRODUCTION

Since old occasions, mushrooms have been utilized in diet valued as food just as the rich hotspot for drugs as well. They have been noted for their limitless bioactive auxiliary metabolites with extraordinary restorative qualities all through the world [1,2,3,4,5]. Mushrooms, heterotrophic life forms that are very explicit in their healthful and biological prerequisites. By and large, they have been classified as humicolous, lignicolous, coprophilous, organisms colous, saprophytic or may show some mycorrhizal relationship with both expansive leaved backwoods trees and gymnosperm taxa. They comprise the most savored food products among the quantity of non-regular groceries basically as a result of their exceptional flavor and surface. Wild palatable mushrooms have been gathered and devoured by individuals for a great many years [6].

Around, among the 14,000 known species, 2,000 were viewed as safe for human utilization and around 650 of them were noted for their important restorative properties [7]. The continuous utilization of mushrooms was fundamentally founded on the three suspicions: first, they are utilized as a feature of the standard eating regimen for their nutraceutical properties like water, minerals, proteins, strands, and starches, with low caloric worth and fat substance [8]. Furthermore, fruiting bodies are additionally refreshing for their delicacy since they are tastefulness enhancers of flavor and fragrance when contrasted with related and different nourishments. Thirdly, mushrooms are broadly utilized for therapeutic purposes. Their pharmacological activity and a remedial interest in advancing human wellbeing have been known for a large number of years [9]. In India, a couple of studies have been done with mushrooms for immunomodulatory properties [10]. A water-solvent glucan was separated from *P. florida* natural product bodies and an examination was likewise completed for its basic portrayal and immunomodulatory impacts [11]. The illnesses effectively recuperating are these days turning into a difficult issue because of the new anti-infection agent's opposition [12]. The relationship between multi-safe microorganisms and clinic contaminations is to be surely featured the issue and there is a pressing need to discover arrangements [13].

The World Health Organization encouraged all the nations to execute control techniques for the engendering of medication multi-safe microbes, featuring the dangers related to the nonattendance of elective treatments against those microorganisms [14] Thusly, examination to discover new antimicrobial substances having success against pathogenic microorganisms impervious to current medications is urgent. The chemical extraction of chloroform, CH3)2CO and water concentrates of the mushroom, Osmoporus odoratus for their antibacterial movement against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and recorded the water extricate alone indicated antibacterial action against the tried living beings and the outcomes were equivalent with that of ampicillin as opposed to chloramphenicol [15].

The high sub-atomic weight substances that are discharged without assimilation and retention from the creature's body are called dietary filaments. Mushrooms contain these substances, which are made out of β-glucan, chitin and heteropolysaccharide (gelatin substances, hemicellulose, polyuronidase, and so on) in the scope of 10-half in the dry load of the substance. Since they assimilate unsafe substances, thwarting their intestinal retention, dietary filaments are successful in forestalling colon and rectal malignancies [16]. To control bacterial sickness in balance fish, shellfish and people generally the chemotherapeutic specialists like chloramphenicol, antibiotic medication, and streptomycin are utilized. There are wide scopes of current anti-toxins that are utilized for the treatment of bacterial diseases yet are still a few difficulties to be required in microbial chemotherapy. One of the issues in the advancement of obstruction of chemotherapeutic specialists is because of the maltreatment of these medications. A few strains of *A. hydrophila* show different medication protections. Some time the chemotherapeutic specialists likewise amass inside different organs of fishes and become harmful to them. In view of the results and the obstruction of microbes work against anti-microbials, as of late much consideration has been paid to extricates and organically dynamic mixes confined from creature and plant source. The multidrug obstruction of certain strains of *A. hydrophila* groups a significant danger in the control of the motile aeromonad septicemia in sea-going creatures and much consideration has been paid to separates and organically dynamic mixes disconnected from plant species

MATERIALS AND METHODS

Collection of Mushroom samples

The fruit bodies of *Agaricus xanthodermus, Agaricus* sp1, *Agaricus* sp2, *Calocybe indica, Calocybe* sp1, *Crinipellis* sp, *Ganoderma lucidum, Ganoderma* sp, *Lentinula sp, Lycoperdon* sp, *Lentinus squarrosulus, Lepiota cristata, Mycena* sp, *Pleurotus cytidiosus, Pleurotus* sp, *Tricholoma* sp1 and *Tricholoma* sp2 were collected from different location of IIT campus, Chennai, Tamil Nadu and were identified in the Centre for Advanced Studies in Botany (CAS), Madras University, Chennai on the basis of microscopic and macroscopic morphological traits with the standard description of [17] (Figure. 1).

Maintenance of mushroom samples

The collected mushrooms were washed thoroughly several times with sterile distilled water. The Inner tissue (trama) was separated aseptically with the aid of a sterile blade. A portion of a small piece of 2 x 2mm of the sterile tissue was then aseptically transferred to the plates of Potato Dextrose Agar (Hi-Media) and maintained for 4 to 5 days. After that, developed mycelium culture was inoculated to 200ml of Potato Dextrose Broth (Hi-Media) with 6mm disc of inoculums from 5 days old plate of culture (Figure. 2). Flasks were incubated at 28°C for 15 days and biomass was separated by filtration and was dried. All transfers were made aseptically. The tissue culture obtained thus was used in subsequent experiments.

Preparation of mycelium mushrooms extract

The dried mycelial biomass was pulverized in a blender. The extraction of the mycelial biomass was carried out using four solvents, ethyl acetate, chloroform, dichloro methane and petroleum ether. For extraction, 500ml of each solvent was dispensed into conical flasks containing 50g of mycelia sample. These were covered with aluminium foil and allowed to stand for 7 days for extraction. The mixtures were filtered using whatman no.1 filter paper and the filtrate was concentrated under a reduced pressure in a rotator evaporator until a semisolid substance was obtained. These were dried inside the crucible under a controlled temperature (45ºC) to obtain a solid extract [18].

Antibacterial screening

Aeromonas hydrophila (IDH1585), bacteria was used for the antibacterial activity obtained from the Microbial Type Culture Collection and the Gene Bank, Institute of Microbial Technology, Chandigarh, India. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37 °C for 24h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and wells of 5 mm in diameter were made aseptically using the well cutter, and 50µL of four different solvent extracts of mycelium were pipette in to the appropriate wells. The stock solutions were prepared at a concentration of 20mg/mL. The Positive control disc containing 30µg of amoxylin, arabinose, bacitrain, chloramphenicol, cloxacillin, methicillin, nystatin, oxacillin, pencilin and streptomycin and a negative control containing 50µL of appropriate solvents were used. The result was calculated by measuring the zone of inhibition in millimetres. For each concentration tested and triplicates were maintained for the confirmation of activity.

Determination of Minimum Inhibitory Concentration (MIC)

The fungal extracts showed significant antibacterial activity were selected for the MIC using micro broth dilution technique [19]. It was performed in 96-well microtiter plates for determining the minimum inhibitory concentration (MIC). Standardized suspensions of the test organisms (*A. hydrophila*) were inoculated into a series of a 96-well microtiter plate. The extracts were diluted to give the final concentrations of 20, 40, 60 and 80 mg/ml of the *A. hydrophila* and the strains were inoculated in tubes with an equal volume of nutrient broth and fungal extracts and incubated at 37 ^{0}C for 24 h. Three control wells were maintained for each strain (media control, organism control and extract control). After overnight incubation, these tubes were observed for turbidity. The lowest concentration (highest dilution) of the extract produced no visible growth (no turbidity) after 24h when compared with the control tubes as an initial MIC. The MIC was determined where growth was no longer visible by assessment of turbidity by optical density readings at 630nm with ELISA read well touch plate (ROBONIC).

DNA fragmentation assay

The genomic DNA fragmentation was observed in the same bacterial (*A. hydrophila*) inoculums, which was used in the micro dilution method. The bacterial inoculums was incubated with ethyl acetate extract of *C. indica* for 3h. The fungal extract (40mg/ml) was predetermined form the micro dilution method, which was chosen for the DNA fragmentation test. Ethyl acetate (negative control) and streptomycin were used as positive control.

The recorded MIC bacterial culture was centrifuged at 5000rpm for 5min to get a bacterial pellet. The bacterial pellet was re suspended in 500µL of solution contained 50Mm Tris, 5Mm EDTA and 50 mM NaCl. Lysozyme was added to a final concentration of 1mg/ml and kept this sample at 55 ^{0}C for 30min. After the incubation period, 10µL of proteinase K (10 mg/ml) and 20 µL of 10% SDS were added and then again incubated at 55 ^{0}C for 10 min or until the solution cleared. The solution was prechilled by ice and the extract was added to an equal volume of phenol, chloroform, and isoamyl alcohol. After that, an equal volume of 4Mm ammonium acetate was added on the supernatant. Then the DNA was precipitated by adding 2 ml of isopropanol or propane- 2, and centrifuged for 10 min. The supernatant was removed after centrifugation and the pellet was washed with 70 % ethanol. DNA pellet was dried and dissolved in 100 µL of TE buffer. Now, the entire genomic DNA was subjected to electrophoresis on 0.8% Agarose (Genei, Bangalore, India) gel at 100 V as constant gel and was stained with ethidium bromide solution (10 mg/ml) and it was documented with the help of gel doc system. Gel image were analysed and interpreted [20].

Statistical analysis

All experimental data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p< 0.05$ was considered for describing the significant level (SPSS Version 20).

RESULTS

Antibacterial Screening

The *invitro* antibacterial studies showed a significant inhibitory activity against the tested bacterium *A. hydrophila* (Table. 1). The inhibition zones produced were significantly ($p<0.05$)

higher for the ethyl acetate extract of *C. indica*, *Tricholoma* sp1, and *G. Lucidum* when compared to the positive control of chloramphenicol and streptomycin. Among the above three mushroom extract, *C. indica* showed the maximum zone of inhibition (16 ± 0.57 mm) against *invitro* growth of *A. hydrophila* when compared to the positive control chloramphenicol (30 ± 0.57 mm). The other two mushroom extract, *Tricholomia* sp1 and *G. lucidum* also showed $P<0.05$ significant inhibitory effect (12 ± 0.57 mm and 8 ± 0.57 mm). The maximum activity of chloroform extract of *G. lucidum* and *C. indica* indicated 4 ± 0.57 mm and 7± 0.57 mm. The maximum activity of dichloromethane extract of *A. xanthodermus* and *Tricholomia* sp1 showed 8± 0.55 and 7± 0.57 mm. The maximum activity of petroleum ether extract of *Lycoperdon* sp1*and Lycoperdon* sp was recorded 6 ± 0.57 mm and 3 ±0.57 mm respectively.

Minimum Inhibitory Concentration

The MICs of tested compounds were in the range of 20 to 80 µL/ ml shown in Table. 2. Among the fungal tested, *C. indica* and *Tricholoma* sp1 showed the highest activity (40µL/ ml and 60µL/m) against *A. hydrophila.* The lowest inhibitory activity was observed in the petroleum ether extract of *L. squarrosulus* (80µl/ml). According to the results of MIC, the ethyl acetate extract of *C. indica* might be used as an antibacterial agent against *A. hydrophila.*

Antibacterial activity of standard antibiotics disc

The antibacterial activity of standard antibiotics disc against *A. hydrophila* is shown in Table.3. The varied resistance and intermediate patterns against the ten antibiotics were noted. Among the ten, maximum resistance patterns were recorded in six. The maximum activity was observed in chloramphenicol (30 ± 0.57mm) and minimum (3 ± 0.57mm) in penicillin

DNA fragmentation assay

The conformation of the suppressive effect of *C. indica* extract on *A. hydrophila* was observed in genomic DNA fragmentation assay. Reason for selecting *A. hydrophila* is very common in the aquatic pathogen, which was used in this study for analyzing the specific immune response and disease resistance in gastropods. From the DNA fragmentation analysis, the ethyl acetate extract of *C. indica* mixed with *A. hydrophila* showed the confirmatory result for DNA fragmentation, when compared to the control (live *A. hydrophila*). Very clear fragmented DNA

band was observed in the tested lane 2 (extract mixed with *A. hydrophila*) and lane 3 (streptomycin with *A. hydrophila*). Fragmentation was not observed in lane 1 (*A. hydrophila* alone) (Figure. 3). This result showed that the ethyl acetate extract of fungal (mushroom) has the ability to minimize or control the growth of bacterial colonies.

DISCUSSION

In a large number of years, the mushroom has been utilized in society medication. Some of them are nutraceuticals (characteristic food having a possible incentive in keeping up great wellbeing and boosting the invulnerable arrangement of the human body) while others can deliver powerful nutriceuticals intensifies that have restorative and healthful traits and are devoured as meds as containers or tablets however not as food [21]. Established researchers, while looking for new remedial other options, has examined numerous sorts of mushrooms and have discovered different helpful exercises, for example, anticarcinogenic, calming, immuno-silencer and anti-toxin. In ongoing many years, the different concentrates of mushrooms and plants have been of incredible premium as wellsprings of common items [22].

The antibacterial activities of aqueous extract of both edible *Pleurotus sajor caju* and *Agaricus* were significant up to 10% dilution against all the tested pathogens. *B. cereus*, *B. subtilis*, *S. aureus* and *S. epidermidis* high sensitivity to metabolic compounds of *L. edodes*, *B. cereus* and *S. aureus* are widely recognized as important food-borne pathogens, and the potential of its inhibition by *L. edodes* may receive more attention [23]. The present study on ethyl acetate extract of *C. indica* (16 ± 0.57 mm) showed more activity against *A. hydrophila* and followed by *Tricholomia* sp1 and *G lucidum* and the minimum activity was observed in dichloromethane extract of *L. squarrosulus* (1 ± 0.57 mm). According to the results of the present study, antimicrobial screening assay, some of the mushrooms studied are potentially a rich source of antimicrobial agents, but many of the mycelia have weak activities. The most active species was *C. indica* and showed broad-spectrum antimicrobial activity.

The ethanol concentrate of *C. indica* was discovered to be compelling in hindering disease development just as moderating and turning around much pathologic status (weakness,

lymphopenia, hepatic harm, and so on) related with the development of malignancy in mice [24]. The antibacterial capability of *C. indica* separate against oral bacterial disconnects. They all around answered to have certain health benefits because of their phytochemical constituents [25]. They were likewise ready to create a wide exhibit of auxiliary metabolites with drug properties. Similarly, portrayed that the nourishing characteristics and cancer prevention agent limits of Pleurotus sp were because of the presence of phenolics, proteins and alkaloids in changing levels [26]. Moreover, researched the antibacterial ascribes of the mycelial extricate from numerous organisms against Gram negative microbes [27]. Extensively, the mycelial biomass of *G. lucidum* separates displayed high inhibitory movement when it was as contrasted and the natural product body extricates, conceivably this may be because of the presence of shifting substance of bioactive atom in the organic product bodies and mycelia [28, 29]. The antibacterial activities of mushroom were due to the presence of bio active components with notable immunomodulatory activities. They have also depicted that the antibacterial activities of mushroom extracts were due to the presence of terpene and polysaccharide [29]. The proximate composition of *Volvariella bombycina* and found 25.5% crude protein in mycelia and 28.3% in fruit body [30]. Antibiotics are not effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately [31]. In the present study a maximum zone inhibition was observed in chloramphenicol (30 ± 0.57 mm) and minimum (3 ± 0.57 mm) in pencillin and these may be good antibiotic in nature against *A. hydrophila.* The minimum inhibitory concentrations (MIC) helps to reveal the amount of antibiotic administration along with that the patient will receive but also the type of antibiotic used to be for this in turn reduces the opportunity for microbial resistance to specific antimicrobial agents. Similarly, the minimum inhibitory concentrations of *Pleurotus citrinopileatus* and *Tricholoma crassum* in active crude extract against *E. coli* and *S. aureus* as determined by the broth micro dilution method [32].

The preliminary screening of the present study ethyl acetate extract of *C. indica* had shown the best activity and was sequentially assayed for its minimum inhibitory concentration (MIC) (40µL/ml). The presence of certain compounds, like ascorbic acid, α-tocopherol, β-carotene and phenolic compounds in this mushroom might be reseasoned behind their antimicrobial attributes. The DNA fragmentation of ethanolic extract of *Cynodon dacylon* mixed

with *A. hydrophila* showed very clear fragmentation were observed and were not observed in bacterial culture alone [33]. Therefore, the present study on the DNA fragmentation assay of the ethyl acetate extract helped to observe the therapeutic potential of the *C. indica*. It thereby gives a fool proof confirmation on the antimicrobial activity of the mushroom extract. In this study, the extract mixed with the bacterial suspension was compared with streptomycin and extract mixed with bacterial culture were observed very clear fragmented DNA band and it showed the potential of *C. indica* in minimizing or controlling the growth of bacterial colonies.

Moreover, this study would help to eradicate many serious problems that arise usually while rearing aquatic organisms. The problem in the aquaculture includes the outbreak of certain dreadful bacterial and protozoan diseases. Currently, antibiotics were used to control such outbreaks to a meager extent. This might be due to the development of multiple drug resistance in such dreadful pathogens of the aquaculture. The *C. indica* is having more therapeutic potential and can be used to control the *A. hydrophila* is an aquatic pathogen causing severe damage in the field of aquaculture. Therefore, this new onsite might enable us to overcome such problems, which are urgent to be resolved.

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CONFLICTS OF INTEREST

No conflict of interest exists.

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Table 1 Antibacterial activity of certain mushroom mycelium extracts against *A. hydrophila*

S. No.	Mushroom species	Ethyl acetate	Chloroform	DCM	Petroleum ether
1	*Agaricus xanthodermus*	2 ± 0.51^{a}	10 ± 1.0^{a}	8 ± 0.55^{b}	-
2	*Agaricus sp1*	-`	2 ± 0.53^{a}	-	3 ± 0.57^{b}
3	*Agaricus sp2*	3 ± 1.0^{a}	-	-	-
4	*Calocybe indica*	16 ± 0.57^{a}	4 ± 0.57^{a}	-	-
5	*Calocybe sp1*	4 ± 0.53^{a}	$3 \pm 1.0^{a.b}$	-	-
6	*Crinipellis sp*	-	-	-	1 ± 0.57^{a}
7	*Ganoderma lucidum*	8 ± 0.57^{a}	7 ± 0.57	-	-
8	*Ganoderma sp*	4 ± 0.57^{a}	3 ± 0.51^{b}	-	-
9	*Lentinula sp*	-	-	-	6 ± 0.57^{a}
10	*Lycoperdon sp*	5 ± 0.57^{a}	3 ± 0.57^{ab}	-	-
11	*Lentinus squarrosulus*	-	2 ± 0.57^{a}	3 ± 0.57^{b}	1 ± 0.57^{c}
12	*Lepiota cristata*	-	-	-	-
13	*Mycena sp*	-	3 ± 0.57^{a}	3 ± 0.57^{b}	$2 \pm 0.51^{c,b}$
14	*Pleurotus cytidiosus*	-	-	-	-
15	*Pleurotus sp*	5 ± 0.57^{a}	2 ± 0.55^{ab}	7 ± 0.57^{c}	-
16	*Tricholoma sp1*	12 ± 0.57^{a}	-	-	-
17	*Tricholoma sp2*	7 ± 1.15^{a}	2 ± 0.51^{b}	-	-

^{abc} Means ± S.D followed by same letters in a colomn for each solvent separately are not significantly different by DMRT at $p<0.05$

Insert Table 2

Table 3 Antibacterial activity of different antibiotic disc against *A. Hydrophila*

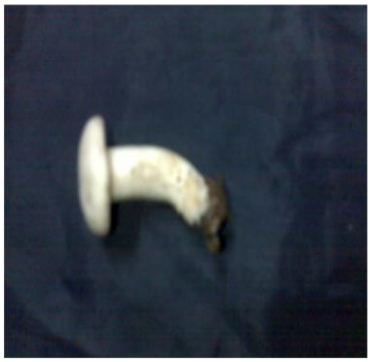
S. No.	Standard Antibiotic (30 µg/disc)	Zone of Inhibition (mm)	Interpretation
1	Amoxylin	15 ± 1.0^{a}	Sensitive
2	Arabinose	-	-
3	Bacitrain	-	-
4	Chloramphenicol	30 ± 0.57^{a}	Sensitive
5	Cloxacillin	-	-
6	Methicillin	-	-
7	Nystatin	21 ± 1.15^{c}	Sensitive
8	Oxacillin	4 ± 1.0^{a}	Intermediate
9	Pencilin	3 ± 0.57^{a}	intermediate
10	Streptomycin	16 ± 0.57^{a}	Sensitive

Figure 1 Wilde Collection of mushroom

Agaricus xanthodermus

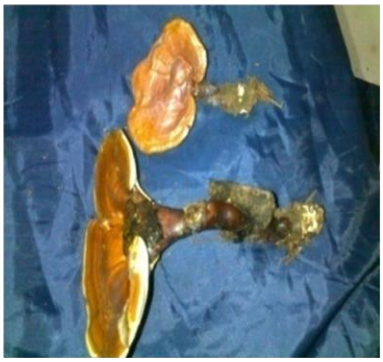
Agaricus sp 1

Agaricus sp2

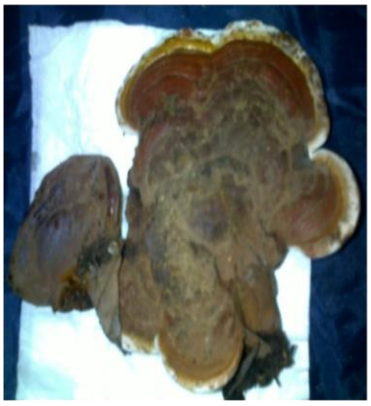


Calocybe indica

Calocybe sp

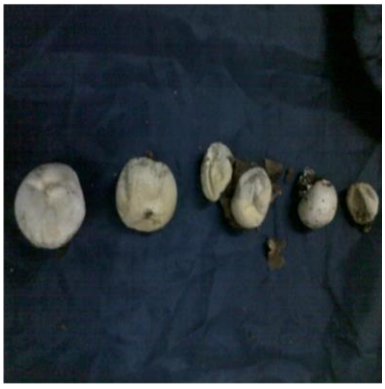


Ganoderma lucidum



Ganoderma sp

Lentinula sp



Lycoperdon sp

Lentinus squarrosulus

Lepiota cristata

Mycena sp

Pleurotus cytidiosus

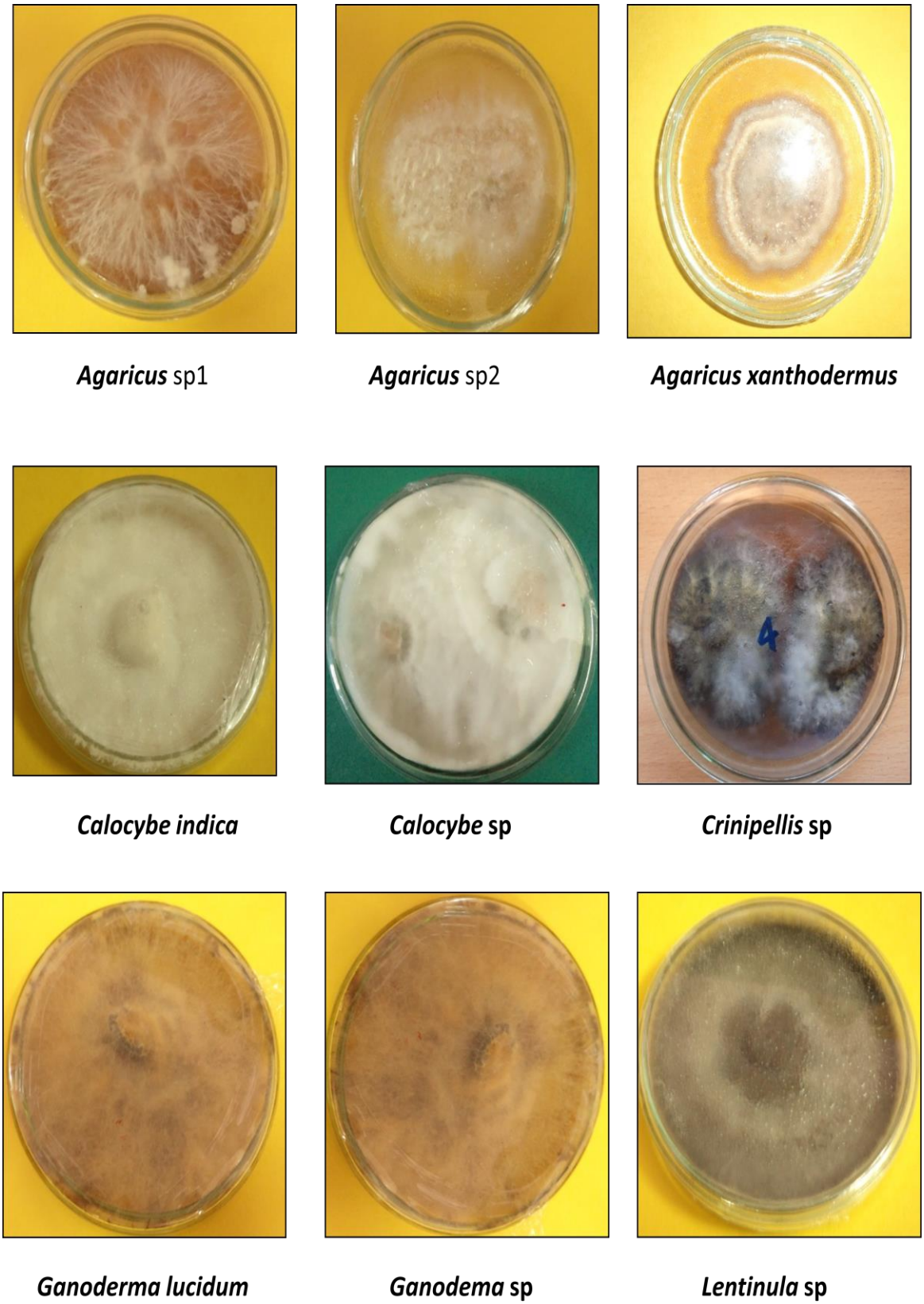
Pleurotus sp

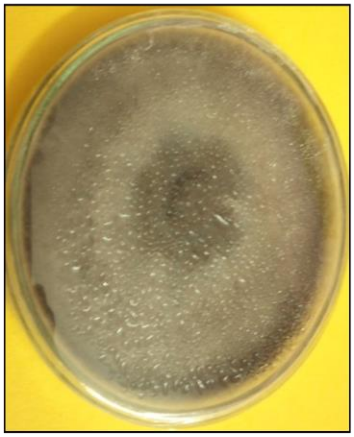
Tricholomia sp1

Tricholomia sp 2

Crinipellis sp

Figure 2 Mycelium culture of different mushroom



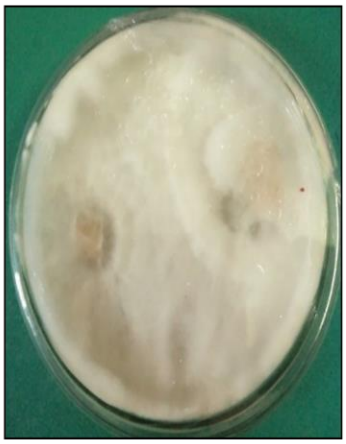


Lycoperdon sp

Lentinus squarrosuls

Lepiota cristata

Mycena sp



Pleurotus cystidiosus

Pleurotus sp

Tricholoma sp1

Tricholoma sp2

Figure 3 Assessment of DNA fragmentation assay of *A. hydrophila*

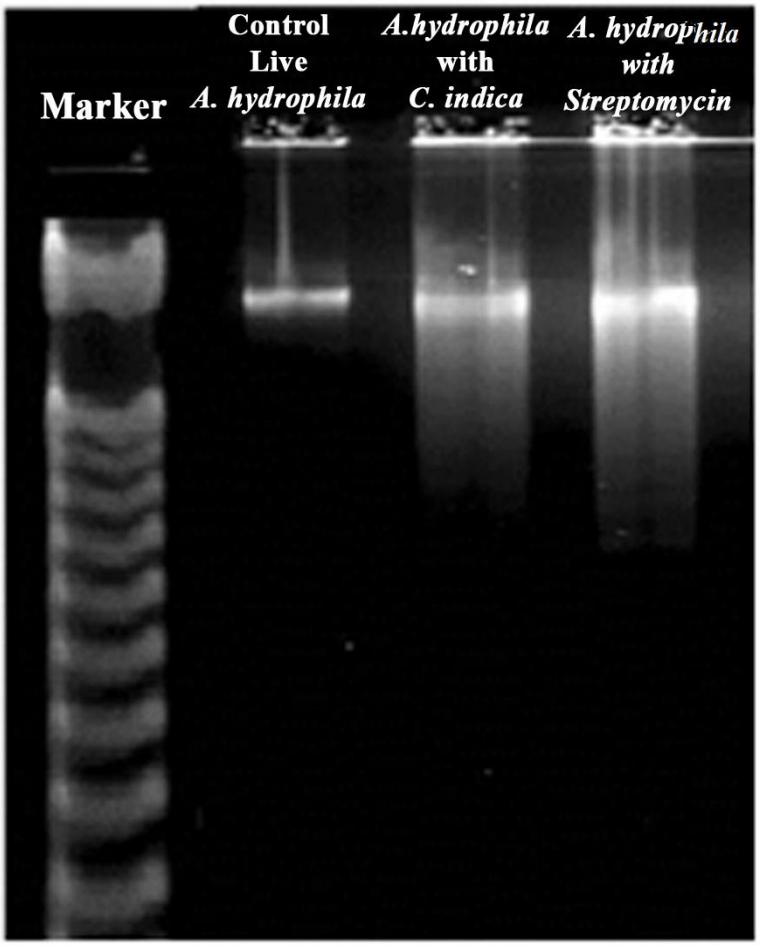


Table 2 **of certain mushroom mycelium extracts against *Aeromonas hydrophila***

S. NO	Mushroom species	Ethyl acetate (µL)				Chloroform (µL)				DCM (µL)				Petroleum ether (µL)			
		20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80
1	*Agaricus xanthodermus*	0.018 ± 0.001^{a}	0.212 ± 0.002^{ba}	0.215 ± 0.001^{ca}	0.221 ± 0.001^{d}	0.117 ± 0.002^{ab}	0.324 ± 0.003^{b}	0.335 ± 0.003^{c}	0.338 ± 0.3^{da}	0.151 ± 0.001^{ac}	0.235 ± 0.2^{b}	0.247 ± 0.003^{c}	0.245 ± 0.002^{dc}	-	-	-	-
2	*Agaricus sp1*	-	-	-	-	0.119 ± 0.002^{ac}	0.125 ± 0.002^{bc}	0.214 ± 0.001^{c}	0.219 ± 0.002^{d}	-	-	-	-	-	-	-	-
3	*Agaricus sp2*	0.079 ± 0.428^{ab}	0.131 ± 0.003^{bc}	0.185 ± 0.001^{c}	0.216 ± 0.001^{d}	0.014 ± 0.003^{a}	0.035 ± 0.181^{ba}	0.101 ± 0.002^{ca}	0.114 ± 0.002^{d}	-	-	-	-	-	-	-	-
4	*Calocybe indica*	0.953 ± 0.006^{a}	1.154 ± 0.003^{bc}	0.807 ± 0.003^{ca}	0.872 ± 0.003^{da}	0.181 ± 0.004^{a}	0.135 ± 0.002^{ba}	0.231 ± 0.002^{c}	0.251 ± 0.002^{da}	-	-	-	-	-	-	-	-
5	*Calocybe sp1*	0.154 ± 0.001^{ac}	0.181 ± 0.002^{bc}	0.205 ± 0.002^{c}	0.221 ± 0.002^{da}	0.355 ± 0.002^{ad}	0.324 ± 0.002^{bd}	0.341 ± 0.003^{c}	0.351 ± 0.002^{d}	-	-	-	-	-	-	-	-
6	*Crinipellis sp*	-	-	-	-	-	-	-	-	0.074	0.132	0.143	0.174	-	-	-	-

										± 0.002[a]	± 0.003[b a]	± 0.005[c a]	± 0.001[d]				
7	*Ganoderma lucidium*	0.157 ± 0.015[a b]	0.343 ± 0.002[b a]	0.489 ± 0.003[c]	0.497 ± 0.002[d a]	0.653 ± 0.032[ac]	0.743 ± 0.022[b]	0.742 ± 0.002[c]	0.816 ± 0.004[d c]	-	-	-	-	-	-	-	--
8	*Ganoderma sp*	0.256 ± 0.002[a]	0.301 ± 0.004[b c]	0.308 ± 0.002[c a]	0.450 ± 0.016[d]	0.135 ± 0.002[a b]	0.147 ± 0.002[b]	0.241 ± 0.004[c b]	0.352 ± 0.003[d]	0.014 ± 0.049[a b]	0.072 ± 0.003[b d]	0.103 ± 0.110[c d]	0.142 ± 0.004[d]	-	-	-	-
9	*Lentinula sp*	-	-	-	-	-	-	-	-	-	-	-	-	0.157 ± 0.001 a	0.207 ± 0.006bc	0.305 ± 0.003 c	0.315 ± 0.001d a
10	*Lycoperdon sp*	0.079 ± 0.003 a	0.153 ± 0.002bc	0.184 ± 0.003c	0.201 ± 0.004da	0.221 ± 0.004ac	0.235 ± 0.002b	0.243 ± 0.004c	0.301 ± 0.003da	-	-	-	-	-	-	-	-
11	*Lentinus squarrosulus*	-	-	-	-	0.205 ± 0.002ab	0.235 ± 0.002b	0.271 ± 0.003cb	0.301 ± 0.004d	-	-	-	0.024 ± 0.003d	0.045 ± 0.004 a	0.073 ± 0.004ba	0.084 ± 0.002 c	0.103 ± 0.003da
12	*Lepiota*	0.206	0.238	0.506	0.509	0.014	0.101	0.104	0.161	-	-	-	-	-	-	-	-

	cristata	± 0.002a	± 0.002bc	± 0.003c	± 0.004da	± 0.001a	± 0.004bc	± 0.003ca	± 0.004d									
13	*Mycena sp*	-	-	-	-	0.073 ± 0.003a	0.085 ± 0.002ba	0.131 ± 0.004c	0.136 ± 0.001da	-		-	-	-	-	-	-	
14	*Pleurotus cytidiosus*	0.134 ± 0.012a	0.158 ± 0.003ba	0.223 ± 0.002ca	0.268 ± 0.007d	-	-	-	-	0.344 ± 0.004a	0.356 ± 0.003cb	0.361 ± 0.002ca	0.368 ± 0.002d	-	-	-	-	
15	*Pleurotus sp*	0.246 ± 0.003a	0.251 ± 0.002ba	0.301 ± 0.003ca	0.316 ± 0.004d	0.184 ± 0.005ab	0.206 ± 0.004bc	0.218 ± 0.005c	0.234 ± 0.004da	-	-	-	-	-	-	-	-	
16	*Tricholoma sp1*	0.406 ± 0.002a	0.472 ± 0.001b	0.853 ± 0.001ca	0.764 ± 0.002d	-	-	-	-	0.131 ± 0.004a	0.152 ± 0.002ba	0.226 ± 0.003c	0.264 ± 0.002da	-	-	-	-	
17	*Tricholoma sp2*	-	-	-	-	0.073 ± 0.004a	0.085 ± 0.002b	0.131 ± 0.002ca	0.136 ± 0.003d	-	-	-	-	-	-	-	-	

[a,b,c,d]Means ± S.D followed by same letters in a column for each solvent separately are not significantly different by DMRT at $p<0.05$