



Journal Homepage: -[www.journalijar.com](http://www.journalijar.com)  
**INTERNATIONAL JOURNAL OF  
 ADVANCED RESEARCH (IJAR)**

Article DOI:10.21474/IJAR01/5473  
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5473>



### RESEARCH ARTICLE

#### ANTIMICROBIAL POTENTIALS OF SPORE CULTURE OF *GEASTRUM* SP., A RARE WILD EDIBLE MUSHROOM OF SIMILIPAL BIOSPHERE RESERVE, ODISHA, INDIA AGAINST SOME SIGNIFICANT HUMAN PATHOGENS.

Mrunmaya K Panda<sup>1</sup>, Hrudayanath Thatoi<sup>2</sup> and \*Kumananda Tayung<sup>3</sup>.

1. Department of Botany, North Orissa University, Takatpur, Baripada, Odisha.
2. Department of Biotechnology, North Orissa University, Baripada, Odisha, India.
3. Mycology and Plant pathology Laboratory Dept. of Botany, Gauhati University, Assam-781014.

#### Manuscript Info

##### Manuscript History

Received: 19 July 2017  
 Final Accepted: 21 August 2017  
 Published: September 2017

##### Key words:-

*Geastrum* sp., Ethnomedicinal uses, Antimicrobial activity, Ethyl acetate extraction.

#### Abstract

A rarely found *Geastrum* species was collected from *Shorearobusta* forest soil of Similipal Biosphere Reserve, Odisha, which is known for its ethnomedicinal uses as wound healing. Fungal mycelia developed from spore culture of the fruiting body were used to evaluate antimicrobial activities. The spore mass of the macro fungi were aseptically inoculated to Potato Dextrose Agar medium to obtain the mycelia. Three different fungal media (*viz.* PDA, MEA, CDA) were used to study the radial growth. Further the fungal mycelium was cultivated in three liquid media (*viz.* PDB, MEB, CDB) and antimicrobial activity of the cultural broth was evaluated at different incubation periods (*viz.* 7 days, 14 days, 21 days) against six bacterial and three fungal clinically significant human pathogens. The crude metabolites of the fungus cultivated in PDB medium was extracted using ethyl acetate as solvent after 14 days of incubation for further antimicrobial and phytochemical analysis. Maximum radial growth of the fungus was observed in Potato dextrose medium and maximum antimicrobial activity was observed in PDB medium as compared to other two media after 14 days of incubation against both bacterial and fungal human pathogens. Qualitative screening of the extract showed presence of phytochemicals. The crude metabolites showed  $\lambda$  max of 1.76 indicating presence of active compounds. The present study demonstrates successful development of mycelia from the macro-fungal spore of *Geastrum* sp., and reports considerable antimicrobial activity of the fungal metabolites which needs to be isolated and characterized.

Copy Right, IJAR, 2017.. All rights reserved.

#### Introduction:-

Mushrooms have been used since long times for the treatments of various diseases, but still it has not been approved in main stream science as drugs or medical treatments (Bensky et al., 1993; Sullivan et al., 2006). The major medicinal properties attributed to mushrooms include anticancer, antimicrobial, antioxidant activities, immune response stimulating effects and blood lipid lowering effects (Bobek et al., 2008). Both fruiting body and mycelia of different mushroom known to contain different compounds such as flavonoids, alkanoids, polysaccharides,

**Corresponding Author:- Dr. Kumananada Tayung**

Address:- Department of Botany, Gauhati University, Guwahati, Assam.

polyglucans, polyphenol, steroids, terpenoids, polyketides and dietary fibers which exert several pharmacological activities (Thatoi et al., 2014).

*Geastrum* are mushrooms of the class Gasteromycetes in which the hymenium is enclosed until spores are matured. This genus has cosmopolitan distribution especially in the sandy soil forests of Asia, Africa, Australia, Europe, Mexico, North America and South America (Nouhra & Toledo 1998, Phosri et al., 2004, Fangfuk et al., 2010). *Geastrum* is superficially similar to *Astraeus*, but it differs in certain characteristics especially have peristome and columella, consists of smaller basidiospores than *Astraeus* and possesses highly branched short capillitial hyphae than *Astraeus* (Phosri et al., 2004). Medicinal uses of *Geastrum* species have not been well documented but some members of this class have been reported to have bioactive potentials (Wasser & Weis, 1999; Guerra Dore et al., 2007). In our earlier study we reported the rare appearance of *Geastrum* species and its ethnomedicinal uses as wound healing by ethnic tribes of Northern Odisha, India (Panda & Tayung, 2015). Further, some species of *Geastrum* have also been reported to have antimicrobial activity from southern India (Chittaragi et al., 2013). Considering the present health scenario of multi-drug resistant pathogenic microorganisms with high mortality, there is an urgent need for new and effective antimicrobial substances to prevent such infections. In this regard, natural resources like mushrooms could be a good alternative as new antimicrobial agents. Therefore, the present investigation was carried out to culture the spore of *Geastrum* species, a wild edible mushroom of Similipal Biosphere Reserve and develop protocol for mycelia culture and to evaluate the *in-vitro* antimicrobial potentials of the mushroom extracts obtained from the culture filtrate against some clinically significant human pathogens.

## Materials and Methods:-

### Sample collection:-

The fruiting bodies of the mushroom species were collected from the Similipal Biosphere Reserve (SBR) of the Mayurbhanj district of Odisha (20°17' to 22°34'N and 85°40' to 87°10'E) covering an area of about 5569 km<sup>2</sup> which forms one of the mega biodiversity zones of the country with rich diversity of flora and fauna. The fruiting bodies were wrapped with sterile foil paper and transported to the laboratory. The identification of the sample was made studying carefully different macroscopic and microscopic characters with the help of standard manuals (Largent & Stuntz 1977; Bilgramiet al., 1979; Purkayastha & Chandra, 1985).

### Sample preparation:-

The fruiting body of the mushroom was surface sterilized by immersing sequentially in 70% ethanol for 3 minutes and 0.5% sodium hypochloride (NaOCl) for 1 minute and rinsed thoroughly with sterile distilled water and air dried. The spore mass of the fruiting body was then aseptically inoculated on to the plates of Potato Dextrose Agar medium. Four replicates were made and the plates were incubated at 30° C for 72 hrs. The plated spores were observed daily for the growth of fungal mycelia. Sub culturing for pure tissue mycelial production was prepared by transferring a small square of 5 x 5 mm from the mother plate culture onto a fresh solid media plate. All transfers were made aseptically under Laminar Air Flow. The tissue culture obtained thus was used in subsequent experiments.

### Determination of fungal growth in different media:-

Radial growth of the macro fungi was determined by placing the agar blocks of pure culture (3mm in diameter) of actively growing culture in Petri-dishes containing Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and CzapekDox Agar (CDA) media. The plates were incubated in an incubator (OSWORLD JRIC-10) and observed daily for its growth. The radial growth of the fungi in respective medium was observed and recorded up to 14 days of incubation.

### Cultivation for metabolite production:-

The mushroom was cultured in three different media namely, Potato Dextrose Broth, Malt Extract Broth and CzapekDox Broth for production of metabolites. Fungal cultures were inoculated in 100 ml conical flasks each of which contained 50 ml of sterile broth mediums (viz. Potato Dextrose Broth, Malt Extract Broth and CzapekDox Broth) and the cultures were incubated by shaking on a rotatory shaker (GeNei SLM-OS-250D) at 140 rpm at room temperature. The cultures were screened for antimicrobial activity at 7, 14 and 21 days after inoculations.

### Preparation of mushroom extracts:-

The macro fungi was cultivated in Potato Dextrose Broth and then incubated at room temperature for 2 weeks by shaking on a rotatory shaker at 140 rpm. Crude metabolites were extracted by solvent extraction method using ethyl

acetate as organic solvent. The solvent extracts were evaporated at 35<sup>0</sup> C by vacuum evaporator and re-dissolved in 15% DMSO solvents to a working concentration of 50 mg/ml and stored at 4<sup>0</sup> C for further study.

#### **Determination of antimicrobial activity:-**

The antimicrobial activity of different broth cultures of the fungus were determined by agar cup diffusion method against six bacterial species namely, *Pseudomonas aeruginosa*(MTCC 424), *Proteus vulgaris* (MTCC 1771 ), *Bacillus subtilis*(MTCC 736), *Staphylococcus aureus*(MTCC 737), *Shigella flexneri*(MTCC 1457), *Klebsiella pneumonia* (MTCC 3384) and three pathogenic fungi *Candida krusei*(MTCC 9215), *Candida albicans*(MTCC 227), *Trichophyton mentagrophytes*(MTCC 8476), as test pathogens obtained from the Institute of Microbial Technology (IMTECH) Chandigarh, India and maintained in the microbial laboratory of Department of Botany, North Orissa University, India. Nutrient agar plates were inoculated with overnight culture of each bacterial suspension. Similarly for the fungal pathogens, Sabouraud Dextrose Agar plates were inoculated with each fungal suspension. The plates with inoculated organisms were evenly spread out with sterile cotton swabs. Agar cups were prepared by scooping out the medium with a sterile cork borer (7 mm in diameter). The cups were then filled with 100 µL of secondary metabolites produced by the fungus in different broth cultures and incubated at 35±1<sup>0</sup> C for 24 hrs observation of zone of inhibition.

Similarly the antimicrobial activity of the crude metabolite extracted (50 mg/ml) was determined by agar cup diffusion method following the same procedures as describes earlier. The agar cups were filled with 100 µL of crude extracts dissolved in 15% DMSO and incubated at 35±1<sup>0</sup> C for 24 hours and observed for the zone of inhibition.

#### **Phytochemical Screening:-**

The crude metabolites obtained from ethyl acetate extraction of the fungus in three different liquid media i.e. PDB, MEB and CDB after 14 days of incubation were subjected to preliminary phytochemical screening for identification of various classes of active chemical constituents using the standard methodology of Harborne (Harborne, 1998). The presence of different phytochemicals in the mushroom species were evaluated as follows: “+” indicates presence of phytochemicals in low amount, “++” moderate, “+++” high, and “-” indicates absence of phytochemicals.

#### **Partial characterization of crude metabolites:-**

For UVVIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No. 1 filter paper. The sample was diluted to 1:10 with the same solvent. To detect the UV-VIS spectrum profile of the crude extract of *Geastrum* sp., the extracts were scanned in the wavelength ranging from 230 nm to 550 nm by using UV spectrophotometer (SPECORD 210) and the characteristic peaks were detected.

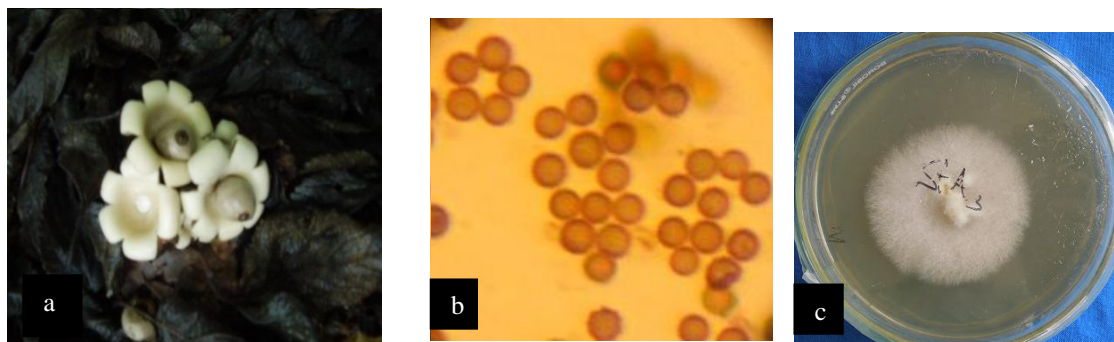
### **Results and discussion:-**

#### **Mushroom collection and identification:-**

In the present study, mushroom species was collected from Satkosia range of Similipal Biosphere Reserve, Odisha, India during (rainy season) July, 2015. The mushroom was found to grow in soil containing leaf litters of Sal (*shorearobusta*) forest. The macroscopic and microscopic character of the mushroom species was studied. The fruiting body is represented by sporophores, which are star shaped epigeous, exoperidium rough 1.5-2.5 mm thick rough, break up outwardly to form 5-8 expanded arms (rays) 2-6 cm across. Rays are glabrous and flame shaped and narrower at tip than the base, white to umber coloured when fresh and brown to black colored at maturity. Endoperidium elevated thin 0.5-1.0 mm, soft, umber to bay brown in colour, surrounded by circular ridge called peristome with a central pore at apex to release spore. Gleba dark brown to almost black in colour, containing millions of spores. Microscopic features revealed that the spores are 3.5-4.5 µm, round, spiny, brownish to yellowish in colour (Fig 1 A). The collected mushroom was thus identified as *Geastrum* sp., by comparing the above characters with other reports (Karun & Sridhar 2014; Boa 2007).

The diversity of *Geastrum* sp has been found to be high in varied geographic locations of Indian subcontinent, but the information on this species has not yet been fully explored. Recently, four *Geastrum* species has been reported from Bamboo leaf litters of Hollongapar Gibbon Wildlife Sanctuary (HGWS), Jorhat, Assam, India (Gogoi & Vipin, 2015). Karun & Sridhar (2014) reported six *Geastrum* species from Western Ghats of Karnataka and west coast of India. A *Geastrum* sp. has also been reported from the Sal forest (*Shorearobusta*) of Khunti forest of Jharkhand during the rainy season (Srivastava & Prabhat, 2014). From earlier reports *Geastrum* sps. are known to

have prophylactic and therapeutic applications in newborn infants (Mooney & Olberchts, 1932) and the mushroom is used to clear the discharges from ear (Robbins et al., 1916). Among the *Geastrum* sps., *G. fimbriatum* known to be edible in India and Madagascar, *G. saccatum* medicinal fungus in Mexico and *G. triplex* is edible and have medicinal use in India and Mexico, and *G. triplex* is also a medicinal mushroom in Guyana (Boa 2007). There has been a report on occurrence of *Geastrum* sp. from Northern Odisha by the author (Panda & Tayung, 2015). Hence, no studies have been made so far on antimicrobial properties of this species, although it has ethno medicinal values.



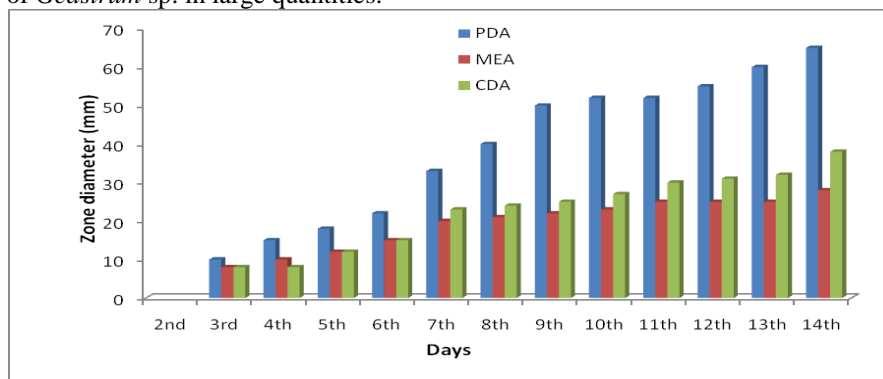
**Fig.1:-** (a) Fruiting bodies of *Geastrum* sp. (b) Basidiospores seen under light microscope (c) mycelia growth on PDA plate

#### Spore culture to obtain mycelia:-

Spores obtained from the fruiting body of the mushroom, *Geastrum* sp. were cultured in Potato Dextrose Agar (PDA) medium in order to obtain the fungal mycelium. Fungal mycelium was obtained from the plated spores of the macrofungi after 6 days of incubation. The mycelia turned white to creamy white after 14 days of incubation (Fig. 1 C). Mainly two methods are being employed for propagation of mushroom; one is spore culture and another is tissue culture. In practice, tissue culturing is regarded as the best method of mushroom cultivation (Oei, 1996). On the other hand spore germination has the disadvantage of taking longer period, with the minute spore size making it relatively difficult to handle (Yu et al., 1984; Oei, 1996). However, in the present investigation, spore culture was established indicating the viability of mushroom spores.

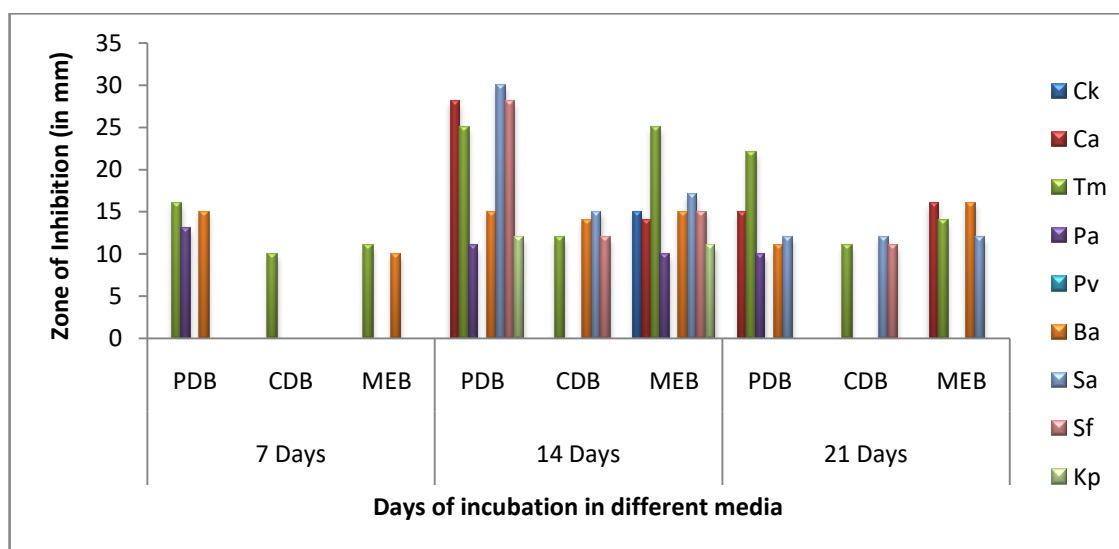
#### Mycelial growth in different fungal media:-

In order to observe efficient *in vitro* growth promotion of the mycelial culture of the macro-fungus, the mycelium obtained by spore culture of *Geastrum* sp. was further inoculated onto three different media namely PDA, MEA and CDA and incubated at BOD incubator at  $30 \pm 1$  °C up to 14 days. It is observed that the mycelia growth starts from 3<sup>rd</sup> day onwards. The results indicated that maximum radial growth was observed in PDA medium and minimum growth was observed at 2% Malt Extract Agar (MEA) medium whereas CzapekDox Agar (CDA) medium showed intermediate growth (Fig. 2). There was steady increased in radial growth of mycelia up to 9<sup>th</sup> days of incubation, thereafter growth slowly decreases. These findings are very much similar with the observations of macro fungal growth of different mushrooms like *Pleurotussajor-caju*, *Pleurotuseryngii*, *Pleurotus columbinus*, *Pleurotussapidus* (Schulzer) and *Xylaria* sp in various culture media (Hasan et al., 2011; Ramesh et al., 2014). The results of the present study are highly promising which suggest the suitability of PDA medium for obtaining mycelial culture of *Geastrum* sp. in large quantities.



**Fig. 2:-** Radial growth of the mycelium of *Geastrum* sp. on different media in different incubation periods.  
**Preliminary screening for antimicrobial activity of mycelial culture in different broth media:-**

Fungal mycelia when grown in broth medium produce secondary metabolites, which have bioactive potentials. Hence, metabolites obtained from the mycelia culture of the spores of the macro-fungus in three different liquid media i.e. PDB, MEB and CDB were evaluated for their antimicrobial activities. The results as shown in Fig.3 indicated that maximum antimicrobial activity was observed in PDB medium followed by MEB and CDB medium. Considerable antimicrobial activity was observed in 14 days of culture in PDB medium followed MEB medium. However, very low antimicrobial activity was observed in 7 days of incubation against most of the test pathogens. Among the different microorganisms the zones of inhibitions were maximum against *Staphylococcus aureus* (MTCC 737) followed by *Candida albicans* (MTCC 227) and *Shigella flexneri* (MTCC 1457) in PDB media after 14 days of incubation. However, persistent inhibitory activity was observed against *Trichophyton mentagrophytes* (MTCC 8476) after 14 days and 21 days of incubation in both PDB and MEB media. Ahmed et al. (2016) studied on nine *Streptomyces* strains and optimized growth conditions during 4 to 10 days of incubation in broth medium for maximum antimicrobial productivity and the strains showed high zone of inhibition in the range of 5-7 days of incubation. Studies made by Kathiresan et al., (2005) reported that 5 days of incubation gave the broad activity of secondary metabolites. Ripa et al., (2009) studied on another *Streptomyces* sp. and observed that antimicrobial metabolites production has started after 7 days of incubation and the highest level of metabolite production was obtained after 10 days of incubation there after the production was declined gradually.



\*Ck-*Candida krusie*; Ca-*Candida albicans*; Tm-*Trichophytonmentagrophytes* ; Pa-*Pseudomonas aeruginosa*; Pv-*Proteus vulgaris* ; Bs-*Bacillus subtilis*; Sa-*staphylococcus aureus*; Sf-*shigella flexneri*; Kp-*Klebsiella pneumoniae*  
 Fig. 3. Zone of inhibition of metabolites produced by mycelial culture of *Geastrum* sp. in different broth media at different time intervals.

#### Antimicrobial activity of ethyl acetate extracts of crude metabolites against some human pathogens:-

Based on the screening of suitable media for antimicrobial metabolite production of the fungus was cultivated in Potato dextrose broth medium extraction of metabolites. The crude metabolites were obtained from ethyl acetate extraction were evaluated for antimicrobial activity against six human pathogenic microorganisms. Considerable antimicrobial activity was observed against the fungal pathogens with highest zone of inhibition against *Trichophytonmentagrophytes* - MTCC 8476 (30 mm) followed by *Candida albicans* - MTCC 227 (28 mm) and *Candida krusie* - MTCC 9215 (18 mm). Out of six test bacterial pathogens higher inhibitory activity was observed against two gram-negative i.e. *Shigella flexneri* - MTCC 1457 (20 mm), and *Klebsiella pneumoniae* - MTCC 3384 (22 mm) and one gram-positive bacteria i.e. *Staphylococcus aureus* - MTCC 737 (22 mm) bacteria (Fig 4). In recent years, many new secondary metabolites have been isolated from higher fungi (Zhong & Xiao, 2009) and are more likely to provide lead compounds for new drug discovery. These metabolites possess the bioactivity such as antimicrobial, immunomodulatory, anticancer, etc. Theonest et al. (2010) investigated antifungal and antibacterial activities of ethyl acetate crude extracts prepared from the fruiting bodies of different developmental stages of edible Tanzanian mushroom *Coprinus cinereus* using agar well method obtaining most bioactivity at post capping stages. Extraction of compounds mainly depends on the polarity and solubility of that compound to the solvent. Thus the

present study results suggest that the active compounds present in the ethyl acetate extracts composed mainly of non-polar ones.

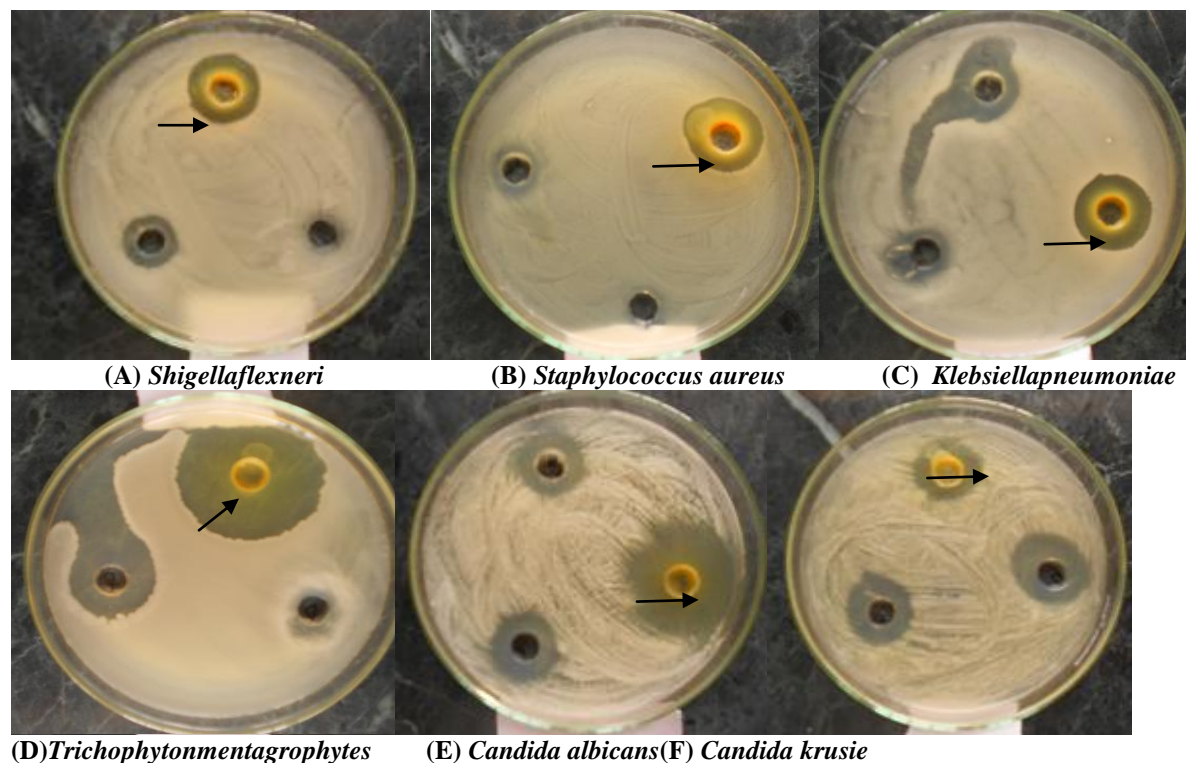


Fig.4. Arrow marks indicate considerable zone of inhibition of the crude metabolites produced by fungal mycelia culture against three bacterial (A,B,C) and three fungal (D,E,F) human pathogens.

#### Preliminary Screening of Phytochemicals:-

The preliminary qualitative phytochemical analysis of the crude metabolites obtained from ethyl acetate extraction of the fungus in three different liquid media i.e. PDB, MEB and CDB showed the presence of active compounds such as phenols, flavonoids, alkaloids, steroids, terpenoids, saponins, carbohydrate, glycoside and tannin (Table 1). Among the studied crude metabolites obtained from different media, crude extracts obtained from PDB media showed the presence of most of the phytochemicals. Recently, the phenolic compounds have attracted much interest among the scientists because various in vitro and in vivo studies have suggested that they possess a variety of beneficial biological properties like anti-inflammatory, antitumor, antioxidant and antimicrobial activities (Lindequist et al., 2005).

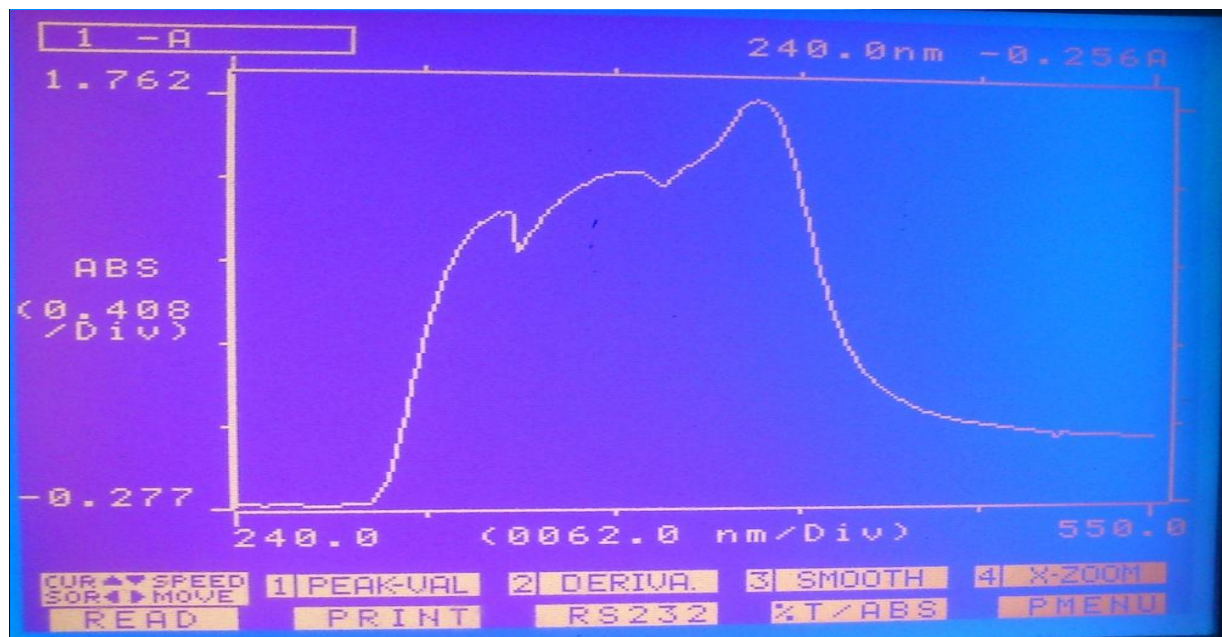
**Table1:-** Qualitative screening of phytochemicals

	PDB	CDB	MEB
Phenol	+++	++	+++
Alkaloid	+	+	-
Flavanoid	++	++	++
Steroid	+++	+++	++
Terpenoid	++	+	++
Saponins	+++	+++	+++
Glycosides	+++	+++	++
Tanin	+	-	-

+ low, ++ moderate, +++ high; and - absent

#### Characterization of crude metabolites:-

The crude metabolite was partially characterized by scanning the metabolites dissolved in ethyl acetate at wavelength from 230 nm to 550 nm in U.V. spectrophotometer (SPECORD 210). The crude metabolites showed a sharp signal ( $\lambda$ -max) with an OD value of 1.76 indicating the presence of bioactive substances (Fig. 5).



**Fig.5:-** UV  $\lambda$ -max in ethyl acetate of the crude metabolites produced by the mycelia of *Geastrum* sp.

Antimicrobial metabolite productions from tissue culture or spore culture of wild mushrooms are being increasingly reported from different parts of the world (Shittu et al., 2005; Hasan et al., 2015; Ramesh et al., 2014). In this study, the fungal mycelia were obtained from the spores of *Geastrum* sp. in the lab environment using PDA medium. Spore germination usually have the disadvantage of minute spore size and taking longer period (Yu et al., 1984; Oei, 1996). However, from this study, it was observed that the spores of *Geastrum* sp. germinated successfully within six days of incubation (fig.1). Different media used in this investigation i.e. PDA, MEA, and CDA showed different mycelia growth and Potato dextrose agar was found to be the best media for the mycelial growth of *Geastrum* species with maximum radial growth after 14 days of incubation (fig.2). This difference of mycelial growth on different agar media may be due to availability of different carbon sources and other required nutrients. The production of antimicrobial metabolite revealed that the antimicrobial activity was high in 14 days of incubation in PDB medium as compared to MEB and CDB media, whereas the activity was shown to be very less during 7 days and 21 days of incubation period in all of the three culture media (fig.3). The fall in activity after 14 days suggests that the active component is chemically unstable or converted to other metabolites. Further, the mycelia of the *Geastrum* sp. was again grown in PDB media for 14 days of incubation and the crude metabolites were extracted using ethyl acetate as solvent. Considerable antimicrobial activity having prominent zone of inhibitions of the crude metabolites (50 mg/ml) were observed against both fungal and bacterial human pathogens. There is also a report on spore germination of *Russala* sp. on Sabouraud dextrose agar plates and the antibacterial metabolites extracted by using ethyl acetate as a solvent found that there is initially increase after 3 days and gradually decrease in activity, which is in line with our study (Shittu et al., 2005). The qualitative screening of phytochemicals showed the presence of active compounds. Extracts obtained from PDB mycelial culture showed the presence of most of the phytochemicals. Antimicrobial activities of the studied mushrooms in most cases were not detected and were very low, that might be due to their lower content of bioactive compounds. The presence of phenolic compounds in the mushroom extracts might also be another reason for the different results obtained in the antimicrobial study. This study showed the pattern of mycelia growth from the spore and antimicrobial metabolite production of *Geastrum* sp. collected from the wild. To the best of our knowledge the antimicrobial metabolite production from the spore culture of *Geastrum* sp. have not been demonstrated so far.

### Conclusion:-

The study exhibited successful development of mycelia culture of the macro-fungal spore of *Geastrum* sp., and reports the antimicrobial activity of the fungal metabolites. Mushroom spores are produced in broth culture of the mycelium in viable condition and developed in to fungal mycelia after 6 days of incubation obtained in PDA medium. The ethyl acetate extract of the macro-fungus, showed significant *in vitro* antimicrobial activity against both bacterial and fungal pathogens. The PDA medium found to be suitable for the mycelia growth and metabolite production to maximize the zone of inhibition in the antimicrobial assay. This indicated that metabolites are broad spectrum in nature and it could be exploited for therapeutic applications. However, a more in depth study is required for isolation and identification of active principle responsible for such bioactivity.

### Acknowledgments:-

We are thankful to the forest staffs of Mayurbhanj Forest Divisions, Baripada for their help and co-operation. This study is a part of project funded by Department of Science and Technology, Govt. of India (Project No. SB/EMEQ-038/2014).

### References:-

1. Guerra DCMP, Azevedo TCG, DeSouza MCR. (2007). Antiinflammatory, antioxidant and cytotoxic actions of  $\beta$ -glucan-rich extract from *Geastrumsaccatum* mushroom. *IntImmunopharmacol* 7: 1160–1169.
2. Ahmed IK, Eltahir HB, Humodi AS. (2016). Streptomyces: isolation, optimization of culture conditions and extraction of secondary metabolites. *IntCurr Pharm J* 5: 27-32.
3. Akyuz M, Kirbag S. (2009). Antimicrobial activity of *Pleurotuseryngii* var. *ferulae* grown on various agro-wastes. *Eur. Asia. J. BioSci* 3:58-63.
4. Bensky D, Gamble A. (1993). Chinese MateriaMedica, 2nd edn. Seattle: Eastland Press.
5. Bilgrami KS, Jamaluddin, Rizi MA.(1979). Fungi of India, Part-I. Today and Tomorrow's Publication, New Delhi, India. 467-469.
6. Boa E. (2007). Wild edible fungi: A global overview of their use and importance to people. New Delhi: FAO (USA) and Daya Publishing House.
7. Bobek P, Galbavy S. (2008). Effect of pleuran ( $\beta$ -glucan from *Pleurotustosreatus*) on the antioxidant status of the organism and on dimethylhydrazine-induced precancerous lesions in rat colon. *Br J Biomed Sci* 58:164–8.
8. Cannon PF, Kirk PM. (2007). Fungal Families of the World. CAB International, UK.
9. Chittaragi A, Naika R, Banakar S, et al. (2013). Phytochemical and Antifungal study of different solvent extracts of *Scleroderma bermudense* Corker. (Sclerodermataceae). *Am. J. Pharm Tech Res.* 3:427-438.
10. Coker WC, Couch JN. (1928). The Gasteromycetes of the Eastern United States and Canada. University of North Carolina Press, Chapel Hill.
11. Cunningham GH. (1944). The Gasteromycetes of Australia and New Zealand.
12. Dring DM. (1964). Gasteromycetes of West Tropical Africa. *Mycol Pap.* 98, 1–60.
13. Fangfuk W, Petchang R, To-anun C, et al. (2010). Identification of Japanese *Astraeus*, based on morphological and phylogenetic analyses. *Mycoscience* 51: 291–299.
14. Gogoi G, Vipin P. (2015). Diversity of Gasteroid Fungi (Basidiomycota) in Hollongapar Gibbon Wildlife Sanctuary, Jorhat, Assam, India. *Curr Res in Environ Appl Mycol* 5: 202–212.
15. Harborne JB. (1998). Phytochem. met. Springer, London.
16. Hasan S, Muhammad AA, Chaudhary MA, et al. (2015). Effects of different culture media, temperature and pH levels on the growth of wild and exotic pleurotus species. *Pak J Phytopathol* 27: 139-145.
17. Hassan AA, Alkurtany AE, Jbara EM, et al. (2011). Evaluation of an Edible Mushroom *Pleurotus* sp. Efficiency against to Plant Pathogens: Nematodes and Soil Fungi. 5th Scientific Conference of College of Agriculture - Tikrit University, Iraq. 431-447.
18. Hibbett DS, Gilbert LB, Donoghue MJ. (2000). Evolutionary instability of ectomycorrhizal symbiosis in basidiomycetes. *Nat.* 40: 506–508.
19. Karun NC, Sridhar KR. (2014). A preliminary study on macrofungal diversity in an arboretum and three plantations of the southwest coast of India. *Curr. Res. Environ. Appl. Mycol.* 4:173–187.
20. Kathiresan K, Balagurunathan K, Selvam MM. (2005). Fungicidal activity of marine actinomycetes against phytopathogenic fungi. *Indian j. Biotechnol.* 4: 271- 276.
21. Largent DL, Johnson D, Stuntz DE, et al. (1977). How to identify mushrooms to genus III, Microscopic features. *Mad. River. Press.* Eureka.



22. Lindequist U, Niedermeyer THJ, Julich WD. (2005). The pharmacological potential of mushrooms. *eCAM* 2:285–299.
23. Lloyd CG. (1902). The Geastreae. *Myc. Wri.* 1: 1–44.
24. Mooney J, Olberchts FM. (1932). The swimmer manuscript, Cherokee sacred formulas and medicinal prescriptions. *Smithson Inst Bur Am Ethnol Bull*99: 1–319.
25. Nouhra ER, DeToledo DL. (1998). The first record of *Astraeushygroetricus* from Argentina. *Mycol.* 12:112–113.
26. Oei P. (1996). Mushroom cultivation. With special emphasis on appropriate techniques for developing countries. TOOL publications, Leiden, Netherlands. 256.
27. Panda M, Tayung K. (2015). Documentation and ethnomedicinal knowledge on wild edible mushrooms among ethnic tribes of northern Odisha, India. *Asian J Pharm Clin Res* 8: 139-143.
28. Phosri C, Martin PM, Walting R. (2013). *Astraeus*: Hidden Dimensins. *IMA Fungus* 4:347–356.
29. Phosri C, Watling R, Martín MP, et al. (2004). The genus *Astraeus* in Thailand. *Mycotaxon* 89: 453–463.
30. Purkayastha RP, Chandra A. (1985). Manual of Indian Edible Mushrooms. Today and Tomorrow's Printers and Publishers, New Delhi, India.
31. Ramesh V, Karunakaran C, Rajendran A. (2014). Optimization of submerged culture conditions for mycelial biomass production with enhanced antibacterial activity of the medicinal macro fungus *Xylaria* sp. Strain R006 against drug resistant bacterial pathogens. *Curr Res Environ Appl Mycol* 4: 88–98.
32. Ripa FA, Nikkon F, Zaman S, et al. (2009). Optimal conditions for antimicrobial metabolites production from a new *Streptomyces* sp. RUPA-08PR isolated from Bangaladeshi soil. *Mycobiol* 37: 211-214.
33. Robbins WW, Harrington JP. (1916). Freire-Marreco B. Ethnobotany of the Tewa Indians. *Smithson. Inst Bur Am Ethnol Bull*.55:1–124.
34. Shittu OB, Alofe FV, Onawunmi GO, et al. (2005). Mycelial Growth and Antibacterial Metabolite Production by Wild Mushrooms. *Afr J Biomed Res* 8:157 – 162.
35. Srivastava AK, PrabhatKFr, Soreng SJ. (2014). Some common wild edible mushrooms growing in Jharkhand. *Int J Sci Environ Tech* 3:577 – 582.
36. Sullivan R, Smith JE, Rowan J. (2006). Medicinal mushrooms and cancer therapy: trans-lating a traditional practice into Western medicine. *Perspect Bio Med* 49:159-170.
37. Thatoi HN, Singdevsachan SK. (2014). Diversity, nutritional composition and medicinal potential of Indian mushrooms: a review. *Afr J Biotech.* 13:523–545.
38. Theonest N, Sylvester LL, Anthony MM. (2010). Antimicrobial activity of ethyl acetate extracts from edible tanzanian *coprinus cinereus* (schaeff) s. gray s.lat. cultivated on grasses supplemented with cow dung manure. *ARPN J Agric Biol Sci* 5: 5.
39. Turkoglu A, Kivrak I, Mercan N, et al. (2006). Antioxidant and antimicrobial activities of *Morchella conica* Pers. *African J Biotechnol* 5: 1146- 1150.
40. Wasser SP, Weis A. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives. *Int J Med Mushrooms* 1: 31-62.
41. Wong JY, Chye FY. (2009). Antioxidant properties of selected tropical wild edible mushrooms *J Food Compost Anal.* 22:269-277.
42. Yu SQ, Trione EJ, Ching TM. (1984). Biochemical determination of the viability of fungal spores and hyphae. *Mycologia.* 76: 608-613.
43. Zhong JJ, Xiao JH. (2009). Secondary Metabolites from Higher Fungi: Discovery, Bioactivity, and Bioproduction. *Adv Biochem Engin/ Biotechnol.* 113: 79-150.