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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF OMAN'S FACULTATIVE *GANODERMA APPLANATUM*.

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Abstract

Ganoderma applanatum is one of the most popular medicinal mushroom due to its various biologically active components. In the present study, we explored the antibacterial effect of Oman's *Ganoderma applanatum*. Methanolic extract obtained from *G. Applanatum* tested for growth inhibition against different bacterial strains (such as *E. coli*, *Klebsiella*, *Staphylococcus* and *Bacillus*) and further we analyzed their chemical profiling by GCMS analysis. We found appreciable anti-microbial activity with different concentration of extract tested. To the best of our knowledge this is the first report on Oman's *Ganoderma applanatum* for its antibacterial and chemical profiling.

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Introduction:-

In different parts of the world, Mushrooms have long been used in traditional medicines and are well known due to their medicinal importance. Around 700 species of Mushroom out of 14000 to 15000 species of mushrooms in the world, have known medicinal properties. However, there are about 1800 species have potential medicinal attributes (Neha et al., 2012; Hawksworth, 2001; Chang and Miles, 2004). In addition, it is also a good source of mineral nutrients. Bacteria are the smallest, unicellular organisms, equipped with all the machinery for growth and self-replication at the expense of foodstuffs. Only a few species of bacteria cause disease, often due to the cell's ability to produce specific poisons, toxins or aggresins (Jain, 2008). Antibacterials are the substances produced by microorganisms, which selectively suppress the growth of or kill other microorganisms at very low concentrations (Neha et al., 2012). Infectious diseases are the major threats to human health. Bacterial infection and reactive oxygen species mediated biological complications remains unresolvable challenges for the past many decades although the effective drugs are available in the market. Commercial antibacterial and antioxidant agents elicit significant toxicity in long term use in particular new bacterial mutants are emerging in response to therapy. These hurdles are major concern for researchers. Finding a new molecules or extract formulation to alleviate this problem is highly warranted. In these scenario, the facultative mushrooms have attracted much attention for the past many decades due their chemical diversity, less toxic nature and abundant source of medicinally important compounds. In particular, a well known *Gonaderma* species has been well enumerated for numerous biological activities.

Although, natural and synthetic antimicrobial agents have been reported to completely restrict the growth of many pathogenic microorganisms. Increasing development of antimicrobial resistance in many pathogens is a danger for public health. Therefore, effective antimicrobial agents are continuously being investigated from different biological

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sources. Many clinically important antibiotics were isolated from fungi belongs to order-*Actinomycetales*. Although, well known examples of antibiotics such as, griseofulvin, cephalosporin and penicillin is produced from different fungi. Since Greek and Roman antiquity, it has been known that macro fungi may be a new source of a useful bioactive compounds (Anke et al., 1989).

In this context, *Ganodermaapplanatum* cultivated in different parts of the world has been shown to be an effective antioxidant and antibacterial agents. However climatic conditions and sessional variation leads to bio synthesis of different molecules with high chemical diversity within them. Bracket shape and reddish brown colour fruit bodies are the characteristic features *Ganodermaapplanatum* (artist's fungus) (Halliwell, 1994). The hard waxy crust has a rough surface (Langseth, 1993), and the dark reddish-brown flesh with a fibrous texture (Halliwell, 1994). Spores are released from pores which are initially white but later turns brownish (Halliwell, 1994). Oman is tropical country with high temperature and we hypothesize that this tropical condition facilitates high accumulation of medicinally important compounds. To address this issue we tested the extract derived from Oman's *Ganodermaapplanatum* on different bacterial strains such as *E.coli*, *Klebsiella*, *Bacillus* and *Staphylococcus*. Similarly, these microorganisms were also tested for antimicrobial activity against the heavy oil derived from Oman's Frankincense, showed strong antimicrobial activity (Hakkim et al., 2015). To the best of our knowledge this the first study of antimicrobial activity and chemical profiling of Oman's *G. applanatum*.

Materials and Methods:-

Extraction of Bio active principles:-

G. applanatum was collected and shade dried for seven days and pulverized by mechanical grinder. Methanolic extract obtained by soxhlet extraction procedure briefly, 20 gm of *G. applanatum* powder was packed in filter paper and placed in soxhlet extractor and 500 ml of methanol (Analytical grade) was used for extraction. Obtained methanolic extract dried at room temperature in fume hood and dried extract store at -20 °C until use for experiments.

Drug preparation:-

Stock solution of *G. applanatum* dried methanolic extract prepared in dimethylsulfoxide (DMSO). Different concentrations such as 10, 25, 50, 75 and 100 mg/ml of *C. schoenanthus* methanolic extract prepared from stock using DMSO and store in -20 °C until use for experiments.

Preparation of test organisms:-

Both gram positive and gram negative bacterial strains: namely *E.coli*, *Klebsiella*, *staphylococcus* and *Bacillus* were used. The test microorganisms were grown on nutrient agar by following standard procedure as described elsewhere.

Antibacterial activity assay:-

The antibacterial activity of *G. applanatum* methanolic extract was determined using agar well diffusion method (Taye et al., 2011). The inoculums were prepared by taking overnight bacterial culture. For sensitivity assay test 38 g of Muller Hinton agar was dissolved in 1000 ml distilled water and autoclaved at 121 °C for 15 min. The media was then poured into sterilized petri-dishes with uniform thickness and the agar was allowed to set at ambient temperature under laminar hood until solidification. These inoculums were spread evenly on the surface of solidified Muller Hinton agar with the help of sterilized spreader. On each plate equidistant wells were made with a 6mm diameter sterilized cork borer. Then 60 µl of different concentration of *G. applanatum* methanolic extract was aseptically added to the respective well. This was followed by allowing the agar plate to stay for 30 min under laminar hood and then incubated at 37 °C for 24 hrs. The formations of clear inhibition zone around the wells were taken as susceptibility measurement.

Gas chromatography mass spectrometry (GCMS) analysis:-

GCMS analysis was done as described earlier (Hakkim et al., 2016). Briefly, GCMS analysis was performed on a Perkin Elmer Clarus 680 GC System, fitted with a Rtx®-5MScapillary column (30m×0.25mm i.d. × 0.25µm film thickness; maximum temperature, 250 °C), coupled to a Perkin Elmer Clarus SQ8S MS. Ultra-high purity helium (99.9999%) was used as carrier gas at a constant flow of 1 ml/min. The injection, transfer line and ion source temperatures were 270, 240 and 240 °C, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The injected sample volume was 1µl with a split ratio of 50:1. The oven temperature

program was 60 °C and accelerated at a rate of 3 °C/minute-240 °C. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST MS20 2011).

Results and Discussion:-

Different types of solvents were used to estimate antimicrobial activities, since they have different polarities in plants. Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane (Lin & Chou, 1984; Yang et al., 2002; Sadaf et al., 2010). The common solvents used for extraction processes are dimethyl sulfoxide (DMSO), acetone, methanol, ethanol and water. Yield and antimicrobial activities of extract are mainly depend on the type of solvent selected (Kagen et al., 1992; Packer and Witt, 1995). *Ganodermasp* provides bioactive compounds that claim to possess antibacterial activity (Djide et al., 2014). In our study, antimicrobial activity of the extract of Oman's *G.applanatum* was determined.

G.applanatum and several other medicinal mushrooms have the prospective to turn into regular drugs. To investigate this potential, defined production processes, standardised quality and methods for its control and legal clinical trials are mandatory (Chang and Wasser, 2012; Lindequist, 2013; Wasser, 2010). These processes require a very close interdisciplinary collaboration, money and time (Ulrike et al., 2015).

The results of our experiment on the antimicrobial activity of Oman's *G. applanatum* shows the 'zone of inhibition' for each bacterial strain with different concentrations as shown in below table.

Table.1

Conc.'s (mg/ml)	Bacterial Strains			
	<i>E.coli</i>	<i>Klebsiellapneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Bacillus Thuringien-sis</i>
Inhibition zone (in mm)				
10	2.75 ± 0.9	4 ± 1.9	4.25 ± 0.9	3.75 ± 0.9
25	12.5 ± 5	4.75 ± 2.1	13 ± 9.2	4.5 ± 0.5
50	4.75±0.9	2.5 ± 0.5	3 ± 0.8	4 ± 1.1
75	5.25±1.2	3 ± 0.8	14.5 ± 1.8	6.75 ± 3.5
100	7± 2.5	3.25± 0.9	11 ± 2.7	9.25 ± 1.4

In our previous study, we found that the presence of γ -terpinene (30.3%), D-Limonene (23.6%), cis-2-methyl-4-pentylthiane-s,s-dioxide (15.3%), β -cymene (12.7%), and α -terpinolene (8.1%) in gonaderma extract (Hakkim et al., 2016). In conclusion, this species (Oman's *G. applanatum*) have efficient antibacterial activity, showing strong zone of inhibition against different bacterial strains tested (Table.1).

References:-

- Anke, H., Bergendorff, O. and Sterner, O. (1989): Assays of the biological activities of guaianesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. *Food Chem Toxicol.*, 27: 393–397.
- Chang, S.T. and Wasser, S.P. (2012): The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *Int. J. Med. Mush.*, 14: 95–134.
- Chang, T. and Miles, G.P. (2004): Medicinal value. *Mushroom cultivation, Nutritional value, Medicinal effect and Environmental Impact.* 2nd edition., 39-51.
- Djide, M.N., Sartini, Latifah, R. and Nursiah, H. (2014): Antibacterial Activity Of Various Extracts From The Fruiting Bodies Of *Ganoderma Lucidum* Growing At Samanea Saman (Jacq.) Merr) Trunk. *International Journal of Scientific & Technology Research.*, Vol. 3, Issue 1.
- Hakkim, F.L., Mohammed, A. and Jackson, A. (2016): Chemical composition and anti-proliferative effect of Oman's *Ganoderma applanatum* on breast cancer and cervical cancer cells. *Journal of Taibah University Medical Sciences.*, 11(2), 145-151.
- Hakkim, F.L., Syed, S.H., Jamal, S. and Mohammed, B. (2015): Chemical Profiling and Anti-Microbial Activity of Frankincense (*Boswellia sacra*) Derived Heavy oil. *Comprehensive Research Journal of Medicine and Medical Science (CRJMMS).*, 3(2): 020 – 024.
- Halliwell, B. (1994): Free Radicals, Antioxidants, and Human Diseases: Curiosity, Cause, or Consequence? *Lancet.*, 344: 721 - 724.
- Hawksworth, D.L. (2001): Mushrooms: the extent of the unexplored potential. *Int J Med Mushrooms.*, 3: 333-337.

9. Jain, N.K. (2008): Bacteria. Pharmaceutical microbiology. Vallabhprakashan., 46-59.
10. Kagan, V.E., Shvedova, A., Serbinova, E., Khan, S., Swanson, C., Powell, R. and Packer, L. (1992): Dihydrolipoic acid--a universal antioxidant both in the membrane and in the aqueous phase. Reduction of peroxy, ascorbyl and chromanoxyl radicals. *Biochem Pharmacol.* 44:1637-1649.
11. Langseth, L. (1993): Form the Editor, Antioxidants and Diseases of the Brain. Antioxidant vitamins Newsletter., 4:3.
12. Lin, J.Y. and Chou, T.B. (1984): Isolation and Characterization of a lectin from edible mushroom, *Volvariella volvacea*. *The Journal of Biological Chemistry.*, 96(1):35-40.
13. Lindequist, U. (2013): The merit of pharmaceutical mushrooms from a pharmaceutical point of view. *Int. J. Med. Mush.*, 15, 517-523.
14. Neha, J., Bharat, P. and Nisha, G. (2012): Comparative Study of Extracts of *Ganoderma Lucidum* for Anthelmintic and Antibacterial Activity. *Am. J. PharmTech Res.*, 2(6).
15. Packer, L. and Witt, E.H. (1995): Antioxidant Properties and Clinical Implications of Alpha-Lipoic Acid. in Packer L. and Cadenas E. eds. *Biothionls in Health and Disease*. New York: Marcel Dekker, Inc., 479-516.
16. Sadaf, Q., Pandey, A.K. and Sandhu, S.S. (2010): Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. *People's Journal of Scientific Research.*, Vol.3(1).
17. Taye, B., Giday, M., Animut, A. and Seid, J. (2011): Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asia Pacific J. Tropical Biomed.*, 1(15):370-375.
18. Ulrike, L., Wolf-Dieter, J. and Sabine, W. (2015): *Ganoderma pfeifferi* – A European relative of *Ganoderma lucidum*. *Phytochemistry.*, xxx, xxx-xxx.
19. Wasser, S.P. (2010): Medicinal mushroom science: history, current status, future trends, and unsolved problems. *Int. J. Med. Mush.*, 12, 1-16.
20. Yang, B.K., Kim, D.H., Jeong, S.C., Das, S., Choi, Y.S., Shin, J.S., Song, S.C. and Song, C.H. (2002): Hypoglycemic effect of a *Lentinusedodesexo*-polymer produced from a submerged mycelial culture. *Bioscience Biotechnology and Biochemistry.*, 66(5): 937-942.