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Screenig of Bioactive Compounds by Gc-Ms From *Fusarium Venenatum*

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Abstract: The present study is carried out to screen bioactive compounds from the major mycoprotein *Fusarium venenatum* .Metabolites were extracted from *Fusarium venenatum* grown in designed medium under optimum condition and the collected filtrate was extracted with organic solvents,concentrated extracts thus prepared from the respective solvent was analyzed by gas chromatography equipped with mass spectroscopy(GCMS).Major Bioactive compounds from all the extracts revealed Phenol, 2,5-bis(1,1-dimethylethy1) and Phthalic acid chloroform: methanol extract revealed the Butenedioic acid, Methanol extract imidazole and flavonoids, Methanol: ethyl acetate: Chloroform extract Hexadecane, Ethyl acetate extract Hexadecanoc acid, Ethyl acetate : Acetonitryl extractTetradecanoic acid. The present study would suggest the possible utilization of the various bioactive compounds for the various food and pharmacological application.

Introduction:

Natural products are chemical compounds derived from living organisms such as plants, animals, and microorganisms.They often have a pharmacological or biological activity and contain many classes of compounds such as terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA). These compounds are important in the field of medicine, agriculture and food preservation. Natural products with antibiotic activity show inhibition on the growth of higher organisms (e.g. tumour cells) or pathogens (e.g. bacteria, fungi, viruses) at low concentration, and subsequently can be used to cure infectious diseases. Secondary metabolites are organic compounds that are produced by different organisms and are not directly associated with their growth, development, or reproduction. Different organisms produce different kind metabolites i.e., Bacteriocin from *Bacillus subtilis*, penicillin from *Penicillium notatum*. Halogenated compounds from marine algae (Maria Teresa Cabrita 2010), caffine, alkaloids and flavonoids from plants, saikosepanins from mammalian cells. Secondary metabolites widely used in medical industries, agricultural fields and in food industries as preservatives. Most of the secondary metabolites are derived from microbial origin. In particular, fungi produce diversified and unique bioactive metabolites, and most of them are medically important antibiotics, including Penicillin, Lovastatin[5], Fingolimod[11], and Caspofungin[6]. *Fusarium venenatum* is a fungal species, which is widely used in the food industry as a protein rich food supplement as well as feed for the livestock worldwide. *Fusarium venenatum* is a micro-fungus belongs to Nectriaceae family used commercially production of mycoprotein in the trade name of 'Quorn' in United Kingdom. *Fusarium venenatum* was chosen at the year of 1960 and after intensive testing the Mycoprotein for 12 years it was approved for sale as consumable by the Ministry of Agriculture[12]. The product is now available in Six European Countries only and the filaments of the fungi were used as mycoprotein which is rich in protein content (44%) and less cholesterol. Prakash et al[7&8] reported that *Fusarium venenatum* has rich protein content can be released by sonication with grinding method, and the protein release constant was 0.680 min. Also he reported that *Fusarium venenatum* has anticancer and antioxidants activity.Microbial metabolites

need to be analyses for food safety perspective; because fungal metabolites are toxic and it will be allergic reaction to human beings. Generally metabolites are two categories i.e., primary and secondary, primary metabolites are large molecules which can be easily separate by numerous methods like centrifugation, filtration and sedimentation. Secondary metabolites are small molecules, which can be separate by extraction methods. Hence, the present study is undertaken to evaluate the potential bioactive compounds from myco-protein *Fusarium venenatum.*

Material and Methods:

Fungal strain

Fusarium venenatum was obtained from Fungal biodiversity centre, Netherland as lyophilized form and the fungi was activated in oats meal medium. Activated fungal culture was maintained on the oats meal agar slant as monosporic culture at 4°C.

Extraction of Crude Metabolites:

For crude metabolite production, fungi was cultivated in jaggery water date extract, K_2HPO_4, KH_2PO_4, $MgSO_4$ media under optimum condition. After seven days of incubation period, the media was centrifuged at 10,000 rpm for 10 minute and the biomass was discarded. Collected supernatant was extracted by Chloroform and methanol (50:50ml), Methanol (100ml), Methanol: Ethyl acetate and chloroform (30:30:40ml), Ethyl acetate (100ml), Ethyl acetate and acetonitrile (50:50ml). The extracted solvents separated by separating funnel and concentrated under rotary vacuum evaporator at 40°C and the concentrated extract was stored in screw cap vial for further studies.

GC-MS analysis

The GC-MS analysis of the extract was carried out using Triple quadrupole mass spectrometer with Fused silica 30mm of length of capillary column, diameter and film thickness is 0.25mm. The coloumn oven temperature is 70 ºC to 300 ºC the coloumn was maintained for 2 to 10 min. Injection port temperature was ensured as 240 ºC and Helium flow rate as 1.5 ml/min. The ionization voltage was EI (-70ev). The samples were injected in split mode as 10. Mass spectral scan range was set at 40-1000 (m/z).Using computer searches on a NIST08s, WILEY8 and FAME MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the extracts were identified.

Results And Discussion:

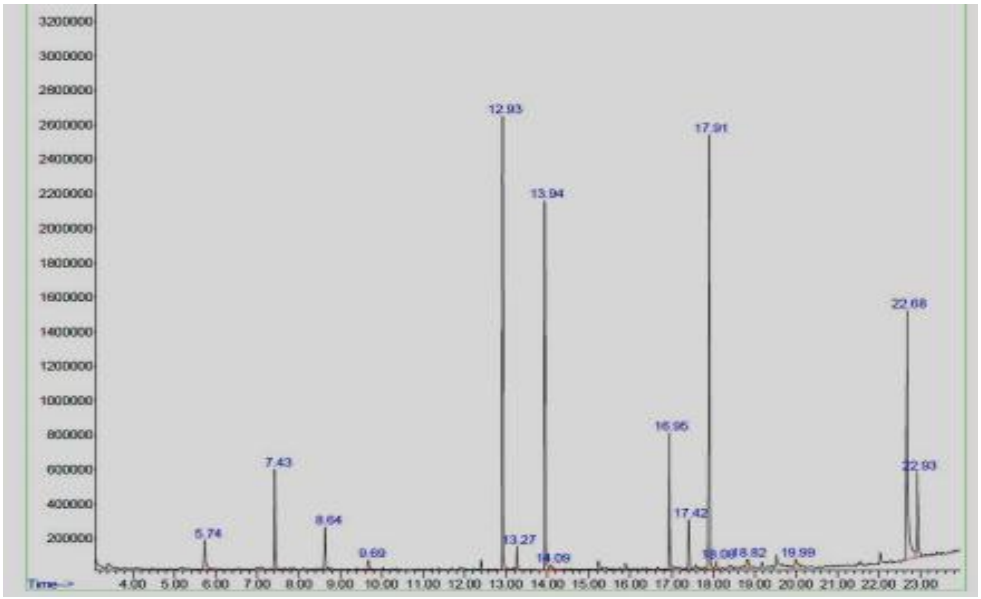

Fig1: GC/MS/MS spectrum of Cholorform:methanol of *Fusarium venenatu*m

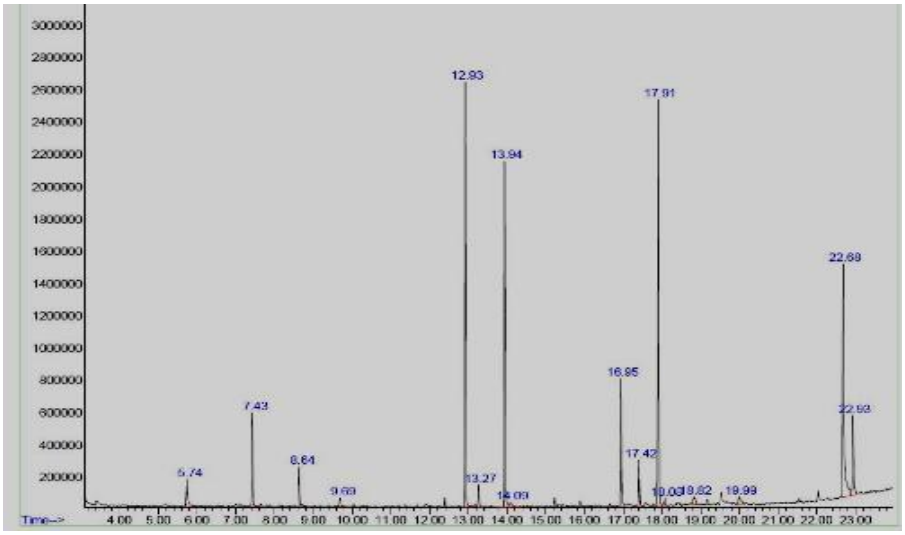

Fig 2: GC/MS/MS spectrum of methanol of *Fusarium venenatu*m

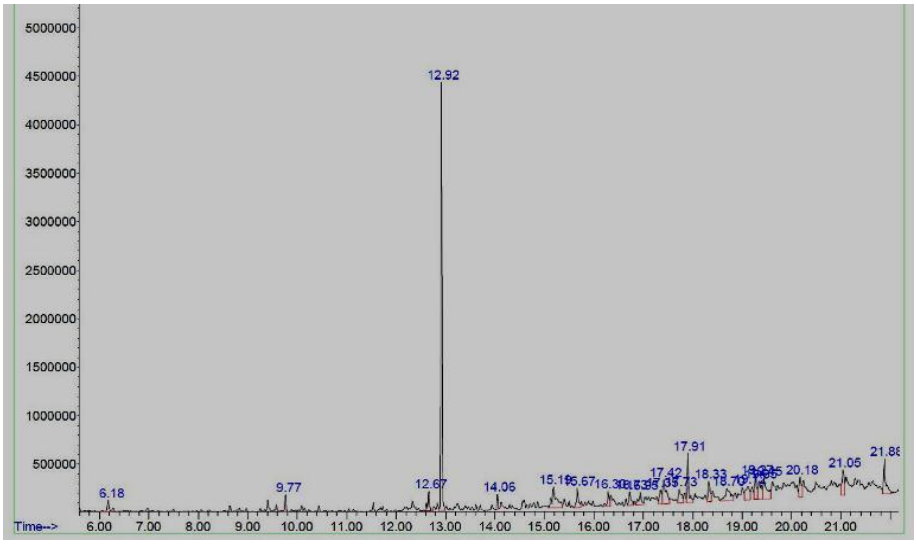

Fig3: GC/MS/MS spectrum of methanol:Ethylacetate:Chloroform of *Fusarium venenatu*m

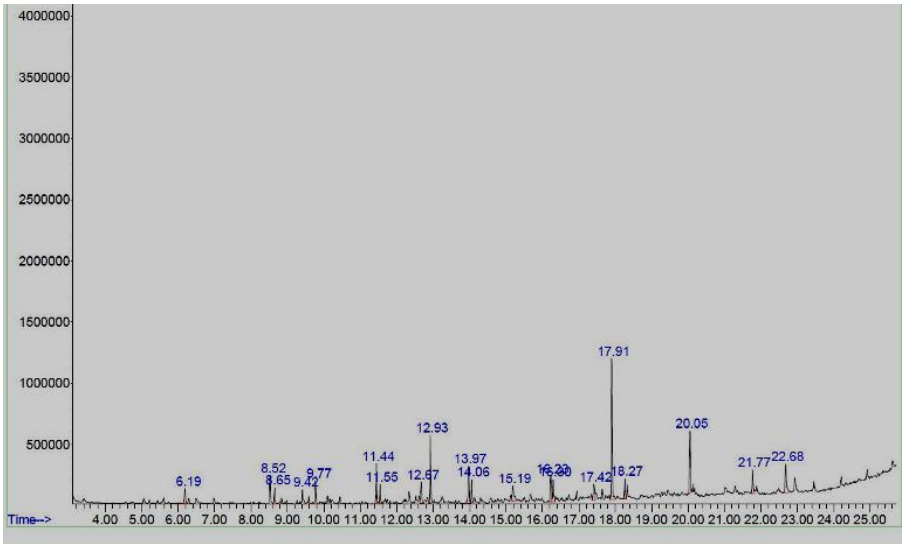

Fig4: GC/MS/MS spectrum of Ethylacetate of *Fusarium venenatu*m

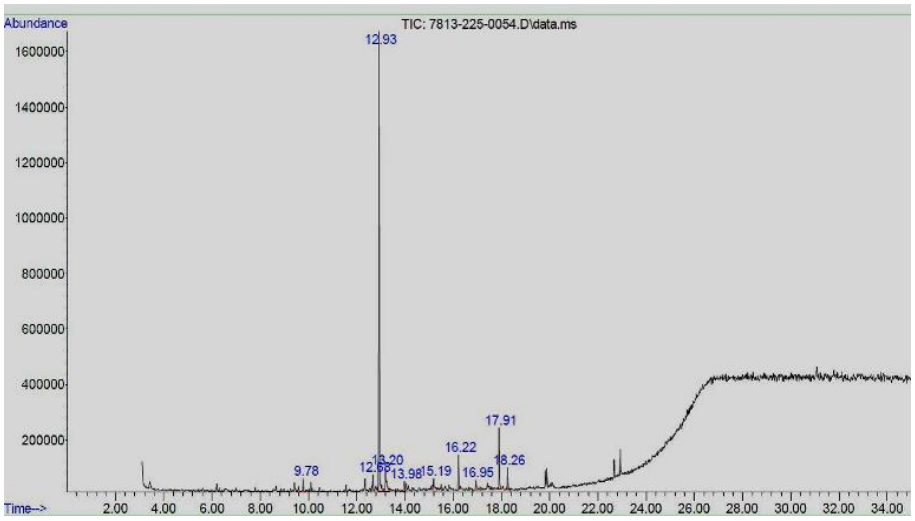

Fig5: GC/MS/MS spectrum of Ethylacetate:Acetonitryl of *Fusarium venenatu*m

The possible bioactive compounds from the culture supernatant of *Fusarium venenatum* has been discussed. Extract was prepared from culture supernatant of fungi grown in jaggery water date extract, K_2HPO_4, KH_2PO_4, $MgSO_4$ media and extracted separately by different solvent system. Diacetoxy scirpenol (DAS) 17.19% (anguidine) has been extracted from Chloroform: Methanol (1:1).It is a mycotoxin from the group of a trichothecenes type. It is a secondary metabolite of *Fusarium sp*. and may cause toxicosis in farm animals. Diacetoxyscirpenol (Anguidine) and its derivates possess anticancer properties. Diacetoxyscirpenol (Anguidine) inhibits initiation of protein synthesis, resulting in the death of rapidly proliferating cells[3]. Pthalic acid 6.11%, is an aromatic dicarboxylic acid also recorded in same solvent system. It is an isomer of isophthalic acid and terephthalic acid. Srinivasan *et al*[11]reported that *Leea indica* (Burm. F) Merr flowers showed phthalic acid esters (95.6%) as major constituents, had good antibacterial and antifungal activity. 2- butenedioic (z) dibutyl esters 1.27%,the other names Maleic acid, dibutyl ester; Butyl maleate; Dibutyl maleate(NIST). it is used in polyesters, and since it is nontoxic, unlike maleic acid, it is used as an acidulant in foods. Phenol, 2,4-bis(1,1-dimethylethyl) 18.10%, similarly[13] reported that Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) identified in the extraction of chloroform from Monochaetia kansensis. Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) has molecular weight, 206; molecular formula,C H O has good antioxidant activity[1]. An isophorone is an α,β-unsaturated cyclic ketone, a colorless to yellowish liquid with a characteristic peppermint-like smell. It is used as a solvent and as an intermediate in organic synthesis. Isophorone 7.43% also occurs naturally in cranberries. Edward *et al.*,[4] reported that isophorone is identified in lemon –lime flavored coconut water. Methanol extracts revealed 21.57% 1H – Imidozole, 11.52% 4H – Pyran and 3.61% cyclohexane carboxylic acid. Imidazole is an organic compound with the formula $(CH)_2N(NH)CH$. The imidazole unit is chemically stable and ubiquitous in biological systems; its proton donor and acceptor moieties easily bind molecules into a dipolar chain[9]. Derivatives of imidazole, called imidazoles is a common family heterocycles with sharing the 1,3-C_3N_2 ring. The imidazole ring is an important building block in biological systems, as exemplified by histidine, histamine and cobalamin[2]. Pyran, or oxine, is a six-membered heterocyclic, non-aromatic ring, consisting of five carbon atoms and one oxygen atom and containing two double bonds. pyrans themselves have little significance in chemistry, many of their derivatives are important biological molecules, such as the pyranoflavonoids. The pyranoflavonoids are a type of flavonoids possessing a pyran group. Cyclohexane carboxylic acid is also known benzoic acid. This is used as antifungal agent and in phenol production. Phenol, 2,4-bis(1,1-dimethylethyl) 29.92% and Phthalic acid 2.17%. were from Methanol + ethyl acetate and Chloroform extract. Ethyl acetate extract shows the presence of Phenol, 2,5-bis(1,1-dimethylethyl) 8.71%, Dibutyl phthalate 17.91, Hexadecanoic acid (8.80%) or palmiticacid. Palmiticacid is a common fatty acids found in animal, plants and microorganisms, and pthalic acid 18.56%. Phenol, 2,4-bis(1,1-dimethylethyl) 65.75% and 4.23% Tetradecanoic acid were extracted from Ethyl acetate and Acetonitryl.

Conclusion:

Fusarium venenatum a major mycoprotein extensively used as protein supplement in the various part of world..A possible bioactive compound has been extracted from the culture filtrate revealed diverse compounds having potential pharmacological activities. Further study will helpful to formulate the possible bioactive metabolites for the diverse pharmacological application.

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