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Bio-Chemical Extraction of Active Compounds of Agaricus (Mushrooms) and it's Antioxidant Activity.

Dr. V. Venkatash gobi[1], Dr. S. Rajasankar[2], Mr. M Ramkumar[3],
Dr. Susie Jeyalyn David[4], Dr. R.Chidambaram[5]
[1] *Phd- Scholar (Medical Anatomy), Bharath University,Chennai.*
[2] *Professor,Vellammal medical college & Hospital,Madurai*
[3]*Tutor, Vellammal medical college & Hospital, Madurai.*
[4]*Assistant Professor,Sree Mookambika Institute if Medical Sciences, Kanyakumari.*
[5]*Director R&D (Medical).Dept. of Radiology, SriLakshminarayana Institute if Medical Scciences,Puducherry.*

Abstract:
Plan: *Aim is to identify the phyto-compounds found in natural food product- Mushrooms(Agaricus) commercially Mother tincture extracted by Sharda Boiron Laboratories Ltd India –Licence no.1703-11.*
Method: *Mode of percolation. To evaluate the anti oxidant level ascorbic acid, α-tocopherol, glutathione, caroteniods, and polyphenol.And oxidative enzymes such as superoxide dismutase(SOD),and catalase(CAT). By Thiocyanate method. Oxidative stress is a major problem for all the biological system including humans. Oxidation is essential to many living organisms for the production of energy to fuel biological processes, excessive production of oxygen derived free radicals is involved in the onset of many pathological conditions such as cirrhosis, metastasis, rheumatoid arthritis, Inflammatory diseases and degenerative diseases of multiple visceras incuuding brain and nerve cells. When degeneration process occurs due to senility, detoriation of normal functions by any diseases the natural antioxidant property in the reduces. To prevent that oxidative cell damage antioxidant food products are essential. Mushrooms have more anti oxidant property.*
Keywords: *Mushroom, Agaricus –extraction of active compounds, thiocyanate method, calculation of drug strength,Anti oxidant activity estimation.*

I. Introduction:

Biological cells, including those of man, animals, and plants, are continuously exposed to a variety of challenges that exert oxidative stress.Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on byoxidative stress, the latter being considered a cause of ageing anddegenerative diseases.Oxidative stress arises in a biological system after an increasedexposure to oxidants, a decrease in the antioxidantcapacity of the system, or both. It is often associated with or leadsto the generation of reactive oxygen species (ROS), including freeradicals, which are strongly implicated in the pathophysiology ofdiseases, such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosisas well as in degenerative processes associated with ageing.

Cells are equipped with several defence systems against freeradical damage, including oxidative enzymes such as superoxidedismutase (SOD) and catalase (CAT), or chemical compounds suchas a-tocopherol, ascorbic acid, carotenoids, polyphenol compoundsand glutathione (Niki, Shimaski, & Mino, 1994). However, antioxidantsupplements or antioxidant-containing foods may be used tohelp the human body to reduce oxidative damage or to protectfood quality by preventing oxidative deterioration (Elmastasa, Isildaka,Reactive free radicals may come from endogenous sourcesthrough normal physiological and metabolic processes such asmitochondrial respiration. Alternatively, they could result from exogenous sources such as exposure to pollutants and ionizingirradiation, and particularly oxygen derived radicals are capableof oxidizing biomolecules, resulting in cell death and tissue damageOxidation is also one of the most important processes of food deterioration since it may affectfood safety, colour, flavour and texture.Turkekulb, & Temura, 2007; Halliwell & Gutteridge, 2003). In recent years, the restriction in the use of synthetic antioxidants,such as BHA (2-tert-butyl-4-methoxyphenol) and BHT (2,6-ditert-butyl-4-methylphenol), has caused an increased interest towardsnatural antioxidant substances (Ames, 1983; Branen,1975). The antioxidants contained in foods, especially vegetables, are phenolic compounds (phenolic acids andflavonoids), carotenoids, tocopherol and ascorbic acid (Cazziet al., 1997; Elmastasa et al., 2007) that are important protective agents for human health (Block, Patterson, & Subar, 1992; Gillmanet al., 1995).Mushrooms are rich sources of those compounds and in the last years we have reported several protocols todetermine their antioxidant activity based on spectrophotometric techniques (Barros, Baptista, Correia, Morais, & Ferreira, 2007; Barros,Baptista,).

Agaricus -Taxonomic position

Division : Mycota
Sub-division :Eumycotina
Class : Basidiomycetes
Subclass : Homobasidiomycetidae
Series :Hymenomycetes
Order : Agaricales
Family : Agaricaceae.
Genus : Agaricus
Species : Bisporus (Fungi, B.R Vashista,S.Chand & Company)

Most widely cultivated species for food purposes is Agaricus Bisporus in India (Rolf Singer 1962).

II. Materials And Methods:

The parenchyma of mushrooms used as tasty material in food has more biochemical and nutritional properties. Less juicy plants mother tincture (extract) is prepared by adding two parts by their weight if alcohol with one part of the plant. (Isildak et al., 2004; Tu¨ rkekul et al., 2004; Manziet al., 1999; Sanmee et al., 2003). Mother tincture is extaracted by the method explained.

1.Ingredients:

a)Cultivated mushroom (part of the fungi)

Agaricus Bisporus include the subclass of Homobasidiomycetidae. The fungi included in this sub-class comprise about 7,500 species which are grouped udder 300 genera.They grow in varied habits such as forest litter, grass land. They are cosmopolitan in their distribution and are best known to every one by their large, consoicuous fructifications called Basidiocarps. Because of the most complexity of the structure Homobasidiomycetidae are called most advanced of all Fungi.

b)Strong alcohol.

Wooden charts, board and knife, porcelain mortar, pestle, Horn made spatula, Linen cloth, clean small beaker, Glass stoppered phial, Another clean phial with new non porus velvet cork, glass funnel with stand filter paper, balance with with weight box.

Procedure:

Fresh Mushroom cut into small pieces with a well polished steel knife on a clean chopping board and pounded to a pulp with mortar and pestle.

Pulp is weighed and taken in a glass jar.Double quantity by weight of strong alcohol is added.

At first powdered is moistened with $1/6^{th}$ part of alcohol.Moistened drug is put into a stoppered bottle and rest of the alcohol added to it.The whole mixture is allowedto stand for 8 days,in a cool_dark place.

After this period the tincture is decanted,strained through the new linen cloth and filtered.Then it is poured in a clean phial provided with a best quality of new,non-porous ,velvet cork.

III. Drug Power, Medicinal Power (Calculation)

Ratio of medicinal substance (Agaricus) : Strong alcohol = 1:2
But in medicinal substance in 1 c.c loss = 2/3 c.c.
∴Net medicinal substance =(1-2/3)c.c .=1/3c.c.
Vehicle in 1c.c. loss = 1/6c.c.
∴Vehicle in 2c.c. loss = 2 x1/6c.c. = 1/3c.c.
∴Net vehicle = (2-1/3) c.c. = 5/3c.c

Net medicinal substance	Solvent/Vehicle (Strong alcohol)	Mother tincture
1/3c.c.	5/3c.c.	(1/3+5/3)c.c. =2c.c.

∴In 2c.c. mother tincture, net medicinal substance =1/3c.c.
In 1c.c. mother tincture, net medicinal substance =1/3x1/2c.c.
∴Drug power (D.P) = 1/6c.c. =1/6c.c.

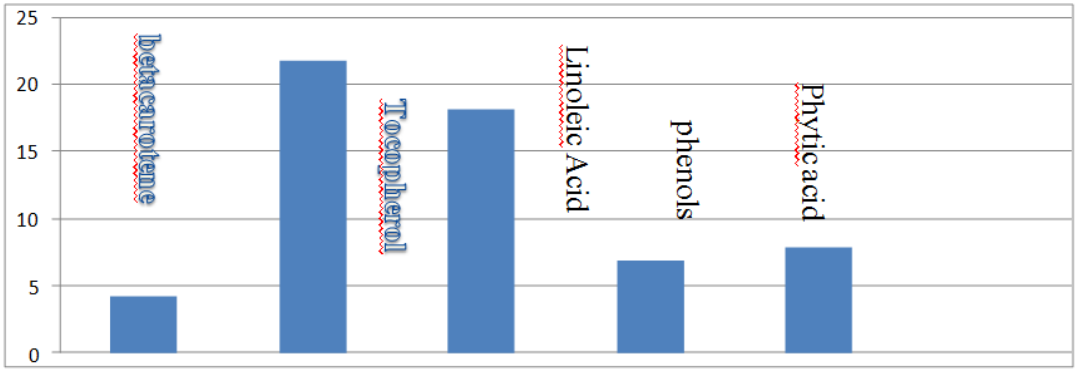
1,1-Diphenyl-2-picryl-hydrazyl (DPPHd), ferrous chloride,polyoxyethylenesorbitan monolaurate (Tween-20), atocopherol,3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) where purchased.

Mushrooms species were divided into seven parts and then air-dried in an oven at 38^{o} C. For methyl alcohol extraction, 20 g of dried mushroom samples was weighed and ground into a finepowder in a mill, then mixed with 200mL methyl alcohol at room temperature at 150 rpm for 24 h. The residue was re-extracted under

the same conditions until the extraction solvents became colourless. The extract obtained was filtered over Whatman no. 1 paper and the filtrate wascollected, then methyl alcohol was removed using a rotary evaporator at 38^{o} C to obtain dry extract. The extract were placed in a plastic bottle and then stored at38^{o} C to prevent oxidative damage until analysis for nearly a week.

IV. Antioxidant Activity Of Dried Specimen Determination

The total antioxidant activities were determined according to the thiocyanate method 10 milligrams of dried extract of each mushroom species were dissolved in 10mL methyl alcohol. Various concerntrations of methanolic extract from mushroom species (50,60,100µg/mL)in 2.5 mL of potassium phosphate buffer were added to linoleic acid 2.5 mL of emulsion in potassium phosphate buffer.The control solution consisted a 5 mL solution consisting of 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04_{M} , $_{p}$H 7.0).The mixed solution was incubated at 40 °C in a glass flask and in the dark.During incubation analysis performed every 6h. After reaction with $FeCI_2$ and thiocyanate, the peroxide values were determined at 5- min intervals by reading absorbance at 500 nm in a spectrophotometeric study.



V. Discussion/ Results:

According to the study several form of edible mushroom species has more amount of medicinal properties. Mushrooms have more anti oxidant property .Oxidative stress is a major problem for all the biological system including humans. Oxidation is essential to many living organisms for the production of energy to fuel biological processes, excessive production of oxygen derived free radicals is involved in the onset of many pathological conditions such as cirrhosis, metastasis, rheumatoid arthritis, Inflammatory diseases and degenerative diseases of multiple visceras incuuding brain and nerve cells. All the organisms are well protected against free radical damage by chemical compounds such as muscarine, ascorbic acid, α-tocopherol, glutathione, caroteniods, and polyphenol.And oxidative enzymes such as superoxide dismutase(SOD),and catalase(CAT). When degeneration process occurs due to senility, detoriation of normal functions by any diseases the natural antioxidant property in the reduces. To prevent that oxidative cell damage antioxidant food products are essential.

Results: Ratio of medicinal substance (Agaricus) : Strong alcohol = 1:2

Mother tincture, net medicinal substance =1/3c.c.

Mother tincture, net medicinal substance =1/3x1/2c.c.

∴Drug power (D.P) = 1/6c.c. (by the method of percolation) .Chemical compounds estimated are muscarine, ascorbic acid, α-tocopherol, glutathione, caroteniods, and polyphenol.And oxidative enzymes such as superoxide dismutase(SOD),and catalase(CAT).

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