IJPSR (2011), Vol. 2, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 June, 2011; received in revised form 16 September, 2011; accepted 27 October, 2011

STUDIES ON PHYTOCHEMICAL AND VITAMIN ANALYSIS OF *PLEUROTUS PLATYPUS* AND *PLEUROTUS OEUS* BY GC-MS AND HPLC TECHNIQUE

G. Sathyaprabha*[1], S. Kumaravel [2] and A. Panneerselvam [3]

Department of Microbiology, PG & Research Department of Microbiology, PRIST University [1], Vallam, Thanjavur, Tamil Nadu, India
Department of Food Quality and Testing, IICPT [2], Thanjavur, Tamil Nadu, India
PG& Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College [3], Poondi- Thanjavur Dt., Tamil Nadu, India

Keywords:
Edible Mushroom,
Phytocomponents,
GC-MS,
Fat soluble vitamins,
Water soluble vitamins,
HPLC

Correspondence to Author:

G. Sathyaprabha

Department of Microbiology, PG & Research Department of Microbiology, PRIST University, Vallam, Thanjavur, Andhra Pradesh, India

ABSTRACT

In this study, *Pleurotus platypus* and *Pleurotus eous* was subjected to identification of bioactive compounds by using Gas chromatography – Mass spectrum technique. These two organisms were extracted with 99% of ethanol. Extracted sample was injected, according to the retention time and peak formation the bioactive compounds are screened. In *Pleurotus platypus* Pyridine-3-carboxamide, 4-dimethylamino-N-(2, 4-difluorophenyl), Piperidin-4-carboxylic acid, Aspidofractinine-3-methanol, (2à, 3á, 5à), Indolizine, and 2-(4-methylphenyl)-. *Pleurotus eous shows that* Imidazolidine, 1, 3-dinitro, Phenol, 2-methyl-4-(1, 1, 3, 3-tetramethylbutyl), Aspidofractinine-3-methanol, (2à, 3á, 5à) and Squalene. *Pleurotus platypus* and *Pleurotus eous* were subjected to analysis the water soluble – Vitamin B and Fat soluble vitamin A, D, E and K was analysed in High Performance Liquid Chromatography technique. According to the results *P.platypus* shows the high content of vitamin E and K.

INTRODUCTION: Today, with the development of better technologies and greater realization of their nutrient values, mushrooms have occupied an important place in food in several parts of the world [1]. Researches on the nutritive value of edible mushrooms indicate that they may be regarded as healthy foods, even though they are deficient in calories and fat and consist of about 90% water [2, 3, 4].

Mushrooms have been reported to be of therapeutic value, useful in preventing diseases such as hypertension, hypercholesterolemia, cancer and also having antibacterial and antiviral properties. These functional characteristics are mainly due to their chemical composition [5, 6, 7, 8]. The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes [9]. While from clinical viewpoint, Bobeck and Galbavy (1999) showed that *P. ostreatus* elicited hypocholesterolemic and antherogenesis inhibition functions in rabbits and rat coutesy of its mycelial secretory products.

However, unlike the fruiting bodies of few other edible mushrooms such as *L. edodes*, *G. fondosa* and *G. lucidium* known for exhibiting antibacterial and antifungal activity *in vitro*, there is lack of information on the microbicidal properties of *P. ostreatus* coupled with inadequate data on its phytochemistry. It is hypothesized that knowledge of the phytoconstituents of *P. ostreatus* would provide an insight into its biological functions beyond nutrition when consumed.

Cultivation and production of edible mushrooms are on the increase, particularly in Europe, America and Asia. Their increased nutritional importance is due to the nutritive value of high-grade mushrooms, which almost equals that of milk [10]. Mushrooms have been evaluated for their nutritional status on the basis of their chemical composition. Cultivated and wild mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, fibres and vitamins [11, 12]. Furthermore, mushrooms are low in calories, sodium, fats and cholesterol [13].

Edible mushrooms have long been considered to have medicinal value and to be devoid of undesirable effects [14]. Most people eat mushrooms, mostly because of its flavor, meaty taste and medicinal value [15]. Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity [14]. These substrates could be used in commercial production of mushrooms for food [15].

Mushrooms are considered as source of proteins, Vitamins, fats, carbohydrates, amino acids, and minerals Kalac, *et.al* (1991), the energy value varies according to species, which is about equal to that of an apple. Many studies gave proof of the fact that some mushrooms species (*Pleurotus* species for example) are useful in some Combinations to cure headache, stomach disorders, colds, fever, asthma and high blood Pressure Kalac, *et al.* (1991), other species are recommended to diabetic and anemic persons, owing to their Low carbohydrate and high folic acid content. Some mushrooms are reputed to possess antiallergic, anti-cholesterol, anti-tumor and anti-cancer properties ITA *et al.*, (2006) & Sesli and Tuzen (1999)

Vitamins are essential nutrients found in foods. The requirements are small but they perform specific and vital functions essential for maintaining health. Fat-soluble vitamins -- vitamins A, D, E and K -- dissolve in fat before they are absorbed in the blood stream to carry out their functions. Excesses of these vitamins are stored in the liver. Because they are stored, they are not needed every day in the diet Alexander (1995).

Water-soluble vitamins dissolve in water and are not stored; they are eliminated in urine. We need a continuous supply of them in our diets. The water-soluble vitamins are the B-complex group and vitamin C. Gershoff (1993).

MATERIALS AND METHODS: 25gm of smashed fresh *Pleurotus eous and Pleurotus platypus* sample was taken in a conical flask with 30ml of distilled alcohol and keep it for overnight soaking then filter the sample and concentrated the sample with help of nitrogen flushing. Filter the filtrate with sodium sulphate. 2ul of purely prepared sample was injected into the programme GC-MS instrument.

GC Program:

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25μm df

Equipment: GC Clarus 500 Perkin Elmer, Carrier gas: 1ml per min, Split: 10:1, Detector: Mass detector Turbo mass gold-Perkin Elmer,Software: Turbomass 5.2,Sample injected: 2μl

Oven temperature Program: 110° C -2 min hold ,Up to 200° C at the rate of 10 ° C/min-No hold,Up to 280 ° C at the rate of 5° C / min-9 min hold,Injector temperature 250° C,Total GC running time 36 min

MS Programme: Library used NIST Version-Year 2005, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450,Solvent Delay: 0-2 min, Total MS running time: 36 min

Water soluble Vitamin- B (HPLC-Shimadzu):

Standard Preparation: Water soluble vitamins - Thiamine, Riboflavin, Pyridoxine, Niacin standard were purchased from Sigma Company, 10mg of standard was dissolved in 10ml of methanol from preparation take 100μl of standard was dissolved in methanol and makeup to 10 ml of methanol. Prepared standard was injected into the HPLC and Fat soluble –Vitamin A, D, E and K sigma standard was dissolved in Mobile phase A and injected the standard in C.18 column.

Sample Preparation: Fresh and healthy *Pleurotus eous and Pleurotus platypus* fruit body was collected and take 500mg of sample and add 1gm of calcium carbonate and add 10ml of mobile phase A (0.5gm of pentane sulfonic acid+0.5gm of Hexane sulfonic acid and 10ml of Acetic acid preparation was makeup to 1000ml with distilled water) kept it in sonicator for 30min at 45^{o}c and make up to 100ml. Mobile phase B (0.5gm of pentane sulfonic acid+0.5gm of Hexane

sulfonic acid and 10ml of Acetic acid preparation was makeup to 1000ml with Methanol)

Fat Soluble Vitamin - A, D3, E and K (HPLC-Shimadzu): Take 1gm of fresh and healthy *Pleurotus eous and Pleurotus platypus* fruit body add 5ml of ethanol and vortex for 10mins. Upper hexane layer was separated and transfer to small test tube and evaporated under nitrogen gas flushing. The residues were dissolved in 50µl of 95% methanol (Mobile Phase: A-Methanol; B-0.02M H_3PO_4 (pH 3.54). Standard preparation: 10mg of standard was dissolved in 1ml of methanol.

RESULTS AND DISCUSSION:

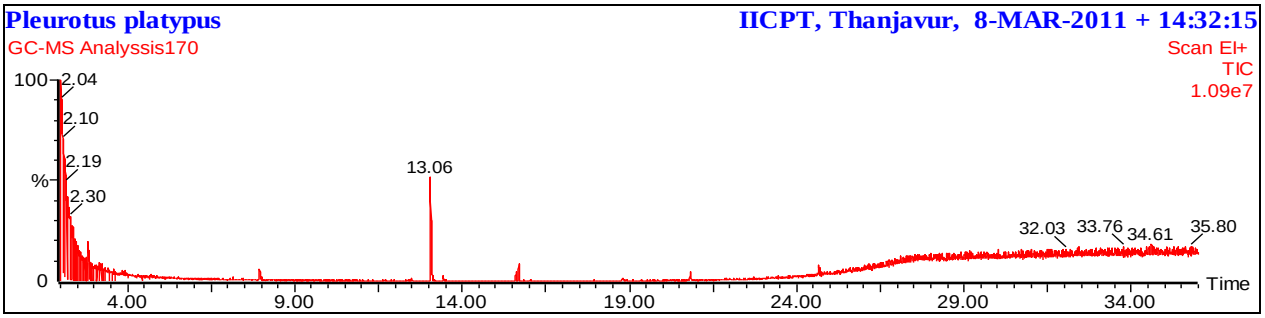
Identification of Components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (**Table 1 & 2**).

TABLE 1: COMPONENTS IDENTIFIED IN THE *PLEUROTUS PLATYPUS SAMPLE* [GC-MS STUDY]

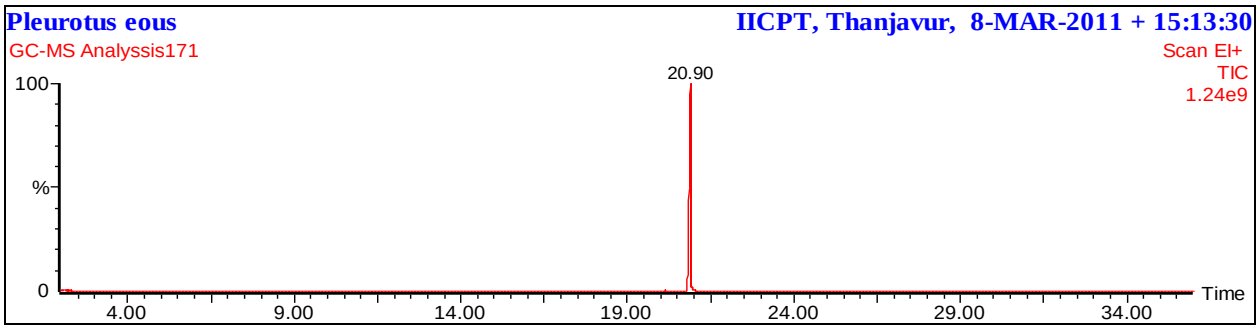
RT	Name of the compound	Molecular Formula	MW	Peak Area %
7.17	DL-Alanine, N-benzoyl-N-(3-chloro-4-fluorophenyl)-, methyl ester	$C_{17}H_{15}ClFNO_3$	335	0.76
7.44	(1H)Pyrrole-2-carboxaldehyde, 4-(trichloroacetyl)-	$C_7H_4Cl_3NO_2$	239	0.76
7.96	2-Amino-4-hydroxy-6, 7, 8-trimethylpteridine	$C_9H_{11}N_5O$	205	3.80
12.49	Benzoic acid 1-methoxy-1H-tetrazol-5-ylmethyl ester	$C_{10}H_{10}N_4O_3$	234	0.76
13.06	Pyridine-3-carboxamide, 4-dimethylamino-N-(2, 4-difluorophenyl)-	$C_{14}H_{13}F_2N_3O$	277	74.14
13.45	a-Ethyl aspartate	$C_6H_{11}NO_4$	161	2.28
18.80	Piperidin-4-carboxylic acid	$C_6H_{11}NO_2$	129	1.52
20.82	Aspidofractinine-3-methanol, (2a, 3a, 5a)-	$C_{20}H_{26}N_2O$	310	4.94
24.67	Indolizine, 2-(4-methylphenyl)-	$C_{15}H_{13}N$	207	11.03

TABLE: 2 COMPONENTS IDENTIFIED IN THE *PLEUROTUS EOUS SAMPLE* [GC-MS STUDY]

RT	Name of the compound	Molecular Formula	MW	Peak Area %
7.96	5-(4-Hexyloxybenzoyloxy)-2-(4-nitrophenyl)pyrimidine	C23H23N3O5	421	0.01
10.15	Imidazolidine, 1, 3-dinitro-	C3H6N4O4	162	0.02
12.64	dl-Alanine	C3H7NO2	89	0.01
16.89	Phenol, 2-methyl-4-(1, 1, 3, 3-tetramethylbutyl)-	C15H24O	220	0.07
17.51	1H-Tetrazol-5-amine	CH3N5	85	0.02
20.90	Aspidofractinine-3-methanol, (2a, 3a, 5a)-	C20H26N2O	310	99.83
24.66	Squalene	C30H50	410	0.05



GC-MS CHROMATOGRAM OF *PLEUROTUS PLATYPUS*



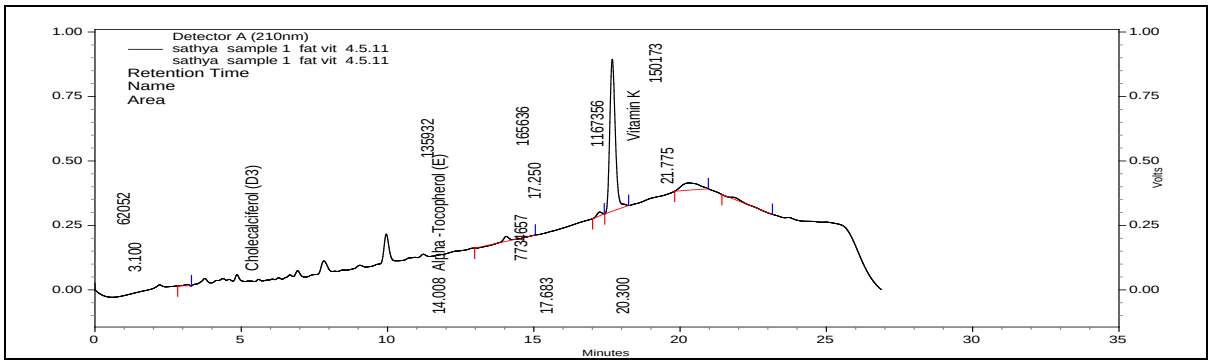
GC-MS CHROMATOGRAM OF *PLEUROTUS EOUS*

P. platypus and *P. eous* was subjected to GC-MS study for identification of medicinal properties, According to the results, the Phytocomponents are screened, and most of the medicinal properties are Pyridine-3-carboxamide, 4-dimethylamino-N-(2,4-difluorophenyl)-Piperidin-4-carboxylic acid, Aspidofractinine-3-methanol, (2a, 3a, 5a)-1H)Pyrrole-2-carboxaldehyde, 4-(trichloroacetyl)-Indolizine, 2- (4- methylphenyl)- are present in *P. platypus*. In *P. eous* Imidazolidine, 1, 3 -dinitro, Phenol, 2-methyl-4-(1, 1, 3, 3-tetramethyl butyl), Aspidofractinine-3-methanol, (2a, 3a, 5a)- and Squalene are presented. The anticancer properties contain Phytochemical compounds were screened in the GC-MS which was shows the high activity, other Phytocomponents were showed in the chromatogram.

Vitamin Analysis-HPLC: Vitamin A, D, E, K are necessary for our day to day life, these are also present in our tested organism *P. platypus* & *P. eous*. According to the result analysis, vitamin Cholecalciferol (D3) and E shows the high level when compared to A and K in *P. platypus*. Vitamin B was analyzed in *P. platypus* was given the high amount of vitamin – B when compared to *P. eous.*

TABLE 3: FAT SOLUBLE ANALYSIS OF *PLEUROTUS EOUS* BY HPLC

Detector A (210nm)						
Pk #	**Retention Time**	**Area**	**Height**	**ESTD concentration**	**Name**	**Units**
				0.000 BDL	Vitamin A (Retinol Acetate)	ug/ul
2	14.008	135932	16815	0.739	Cholecalciferol (D3)	ug/ul
5	20.300	1167356	28094	0.233	Alpha -Tocopherol (E)	ug/ul
6	21.775	150173	4744	0.146	Vitamin K	ug/ul



According to the results, vitamin A concentration shows the below detected level in both *Pleurotus eous* and *Pleurotus platypus*, vitamin D3 is higher in *Pleurotus eous* while it's absent in *Pleurotus platypus*. Vitamin E (0.617µg/µl) and vitamin K (0.900µg/µl) level in *Pleurotus platypus*

TABLE 4: FAT SOLUBLE VITAMIN ANALYSIS IN *PLEUROTUS PLATYPUS* BY HPLC

Detector A (210nm)						
Pk #	**Retention Time**	**Area**	**Height**	**ESTD concentration**	**Name**	**Units**
				0.000 BDL	Vitamin A (Retinol Acetate)	ug/ul
				0.000 BDL	Cholecalciferol (D3)	ug/ul
6	19.992	3090484	83219	0.617	Alpha -Tocopherol (E)	ug/ul
7	21.950	925689	16839	0.900	Vitamin K	ug/ul

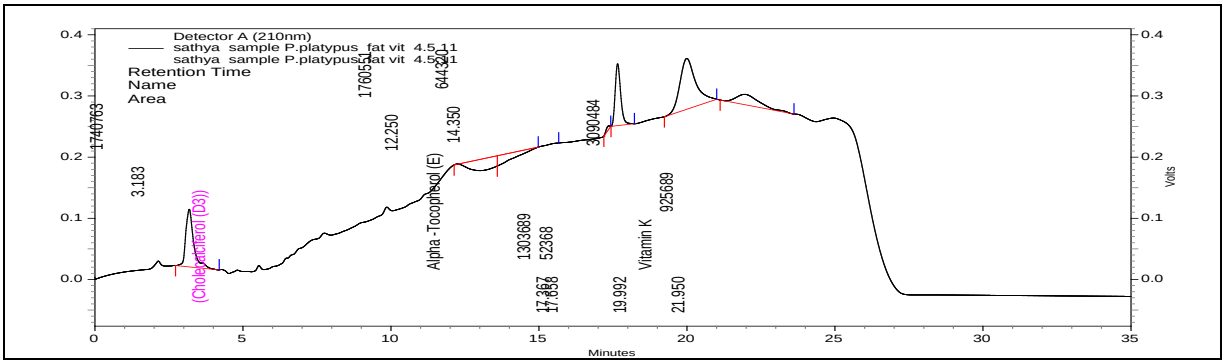


TABLE 5: WATER SOLUBLE VITAMINS IN *PLEUROTUS EOUS* BY HPLC

Detector A (285nm)						
Pk #	**Retention Time**	**Area**	**Height**	**Name**	**ESTD concentration**	**Units**
2	8.925	585	42	NIACIN	0.043	ug/ul
4	11.883	786	63	PYRIDOXINE	0.010	ug/ul
5	23.433	33	6	THIAMINE	0.011	ug/ul
6	26.058	2	3	RIBOFLAVIN	0.012	ug/ul

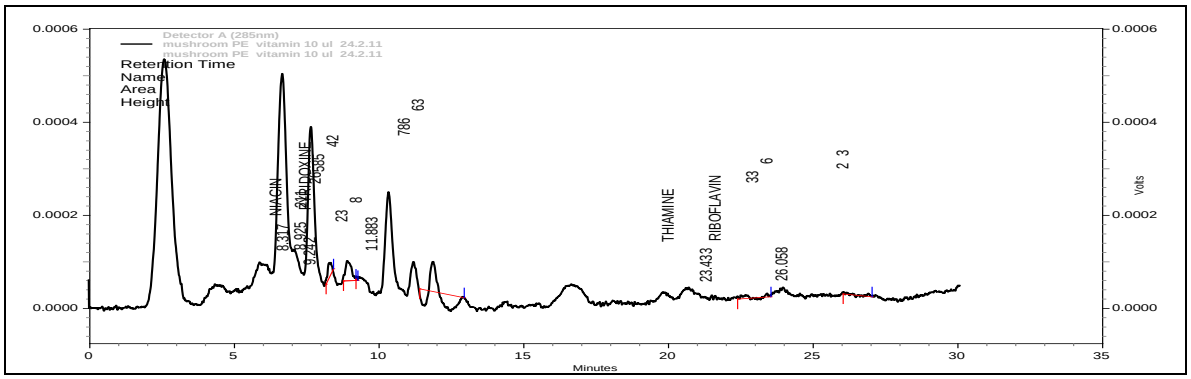
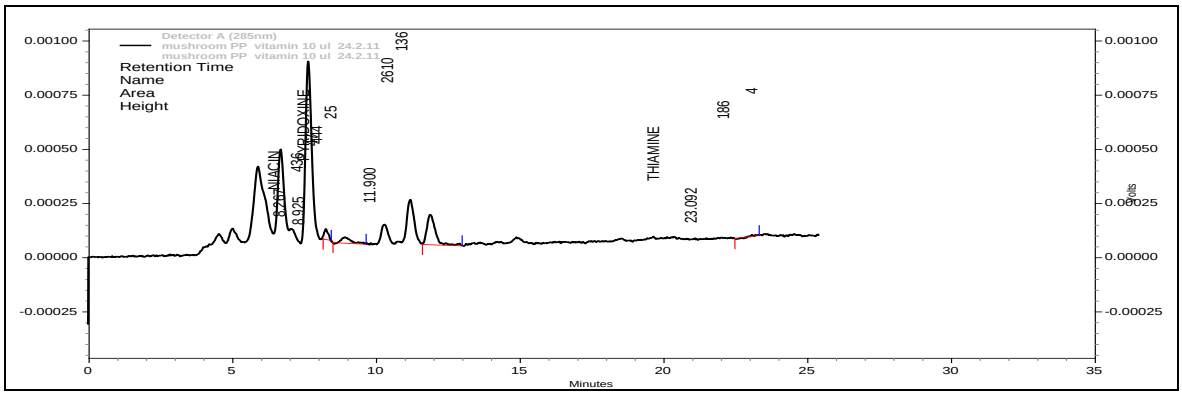


TABLE 6: WATER SOLUBLE VITAMINS IN *PLEUROTUS PLATYPUS*

Detector A (285nm)						
Pk #	**Retention Time**	**Area**	**Height**	**Name**	**ESTD concentration**	**Units**
2	8.925	444	25	NIACIN	0.037	ug/ul
3	11.900	2610	136	PYRIDOXINE	0.015	ug/ul
4	23.092	186	4	THIAMINE	0.020	ug/ul
				RIBOFLAVIN	0.000 BDL	ug/ul



CONCLUSION: In this study, *Pleurotus platypus* and *Pleurotus eous* were extracted with pure ethanol and subjected to screening of bioactive compounds by Gas Chromatography-Mass Spectrum technique, according to the results various active compounds are presented in Pleurotus platypus when compared with Pleurotus eous. The active compounds are Pyridine-3-carboxamide, 4- dimethylamino-N- (2, 4- difluoro-phenyl)-, Piperidin-4-carboxylic acid, Aspidofractinine-3-methanol, (2a, 3a, 5a)-, 1H) Pyrrole-2-carboxaldehyde, 4-(trichloroacetyl)-, Indolizine, 2-(4-methylphenyl)- are present in *P. platypus*. In *P. eous* Imidazolidine, 1, 3-dinitro, Phenol, 2-methyl-4-(1, 1, 3, 3-tetramethylbutyl), Aspidofractinine-3-methanol, (2à, 3á, 5à) - and Squalene.

Water soluble vitamins and Fat soluble vitamins were analyzed in HPLC, results were noted in the above table, results shows that vitamin A was shows the below detectable level in *P.platypus* and *P.eous* but vitamin D3 was shows the maximum level (0.739 ug/ul) in *P.eous* while it absent in *P.platypus.* Vitamin K (0.900 ug/ul) was highest in *P.platypus* when compared to vitamin E (0.617ug/ul) in *P.platypus* .Water soluble vitamins (Niacin 0.043ug/ul,) in *P.eous* while in *P.platypus* (Niacin 0.37 ug/ul,) while Pyridoxine, Thiamine and Riboflavin was show that low level in both the species *P.platypus* and *P.eous*.

ACKNOWLEDGEMENT: I thank to my Guide, Dr. A. Panneerselvam, A.V.V.M. Sri Pushpam College, Co-

Guide, Dr. P. Prabhakaran, PRIST University-Vallam, Dr.Usha K.N. Government Arts and Science Women's College, Thanjavur. The Director, IICPT-Thanjavur, Shri. S. Kumaravel, Scientist, IICPT- Thanjavur and Mr. R. Paranthaman, Technical Assistant, IICPT, Dr. P. Karthikeyan, Scientist PRIST University, Thanjavur for helping me to carry out my research work and to publish.

REFERENCE:

1. Hafiz, F., M. Begum, S. Parveen, Z. Nessa and A.K. Azad, 2003. Study of edible mushroom grown on Eucalyptus Camaldulensis trunk and under the soil of Albizzia Procera. *Pakistan J. Nutr.*, 2: 279–82
2. Nylen, B., 1985. *Vara Matsvampar.* Naturoch. Kultur/LTs forlag
3. Manzi, P., L. Gambelli, S. Marconi, V. Vivandti and L. Pizzoferrato, 1999.Nutrients in edible mushrooms: An interspecies comparative study.*Food Chem*., 65: 477–82
4. Sanmee, R., B. Dell, P. Lumyong, K. Izumori and S. Lumyong, 2003.Nutritive value of popular wild edible mushrooms from Northern Thailand. *Food Chem.,* 82: 527–32
5. Cochran, K.W., 1978. Medicinal effects. *In*: Chang, S.T. and W. Hayes (eds.), *The Biology and Cultivation of Edible Mushrooms,* Pp: 169–87. Academic Press, New York
6. Chovot, V., L. Opletal, L. Jahodar, A.V. Patel, C.G. Dacke and G. Blunden,1997. Ergota- 4, 6, 8, 22 -tetraen- 3-one from the edible fungus *Pleurotus ostreatus* (oyster fungus). *Phytochem.,* 45: 1669–71
7. Gunde-Cimerman, N., 1999. Medicinal value of the genus *Pleurotus* (Fr.) P.Karst (Agaricale s.l, basidiomycetes.). *Int. J. Med. Mush.,* 1: 69–80
8. Manzi, P., A. Aguzzi and L. Pizzzoferrato, 2001. Nutritional value of Mushrooms widely consumed in Italy. *Food Chem*., 73: 321–5
9. Fountoulakis MS, Dokianakis SN, Kornaros ME, Aggelis GG, Lyberatos G (2002). Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. Water Res, 36: 4735-4744.
10. Bobek P. Galbavy S (1999). Hypocholesteremic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbits Nahrung 43: 339–342.

****