International Journal of Drug Development & Research
| January-March 2013 | Vol. 5 | Issue 1 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.13 & H index 2

Full Length Research Manuscript

Analysis on total Anti-Oxidant activity of *Pleurotus eous* Mushroom Correlated to its Phenolic and Flavonoid Content

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Abstract

In the present study total antioxidant activity of crude extracts of mushroom *Pleurotus eous* and its phenolic, flavonoid content have been investigated. The study was carried out by preparing methanol, ethyl acetate, aqueous and pet ether extracts. The results showed that methanolic extract of *P.eous* has higher antioxidant capacity (88, 56 and 34 mg equivalents of BHT/100 mg of MeOH, EA, PE extracts respectively) phenolic content (354.59, 189.37, 146.38 and 142.98 mg/gm of dry mass of MeOH , EA, PE, Aqs extracts respectively), and flavonoid content (in terms of Quercetin equivalent as 334.785, 257.60, 152.173 and 93.48 mg/gm of dry mass of MeOH, EA, Aqs, PE extracts respectively) than ethyl acetate, petroleum ether and aqueous extracts. The total antioxidant capacity of mushroom *P.eous* is in good correlation with their phenolic and flavonoid content. The study describes that the methanol could be the good solvent for the isolation of potent phenolic and flavonoid compounds present in this mushroom species. The detailed investigation of methanol extract confirmed the presence of poly-phenols such as flavonoids and tannins which are well known natural antioxidants. Thus, the antioxidant potential of extracts of *P.eous* is confirmed due to the presence of polyphenolic compounds.

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Key words:
Aluminium trichloride method, phosphomolybdenum assay, FC assay.

How to Cite this Paper:
Suseem S. R, Mary Saral. A "Analysis on total Anti-Oxidant activity of *Pleurotus eous* Mushroom Correlated to its Phenolic and Flavonoid Content" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 174-178.

Article History:----------
Date of Submission: 12-12-2012
Date of Acceptance: 28-12-2012
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

Phenolic compounds make up one of the major families of secondary metabolites in mushrooms, and they represent a diverse group of compounds. Phenolics can be broadly divided into non-soluble compounds such as condensed tannins, lignin, cell-wall bound hydroxy cinnammic acid and soluble

phenolics such as phenolic acids, phenyl propanoids, flavonoids and quinones. All these groups are involved in many processes in plants and animals. The flavonoids, is of particular interest because of its multiple roles in plants and its impact on human health [1].

The flavonoid family is subdivided into different sub-families such as flavones, isoflavones, flavanones, flavonols, anthocyanins, chalcones and condensed tannins. All of these types of compounds comprise three aromatic rings harboring different substitutions such as methylation and hydroxylation. Flavonoids can be easily extracted with polar solvents such as methanol, which is not the case for insoluble lignins and tannins that bind to proteins on cell disruption during the extraction.

Flavonoids, as established natural anti-oxidant, have captured a fast growing interest among consumers and scientists in medical, pharmaceutical, chemical and agricultural industries. They are a group of poly phenolic compounds diverse in chemical structure and characteristics, found ubiquitously as secondary metabolites or chemical constituents in plants either consumed as foods or used by man in folk-lore medicine. They occur naturally in fruit, vegetables, nuts, grains, seeds, flowers, roots, stems and bark of plants; and are integral part of the human diet [2].

Mushrooms are reported to contain relatively large amounts of vitamins A, C and of β–carotene, all of which have protective effects because of their antioxidant properties. Ethyl acetate and methanol extracts of *Pleurotus florida* have been found to exhibit potent scavenging of hydroxyl radicals and inhibition of lipid peroxidation activities [3]. Up till now, research has tended to focus on the dietary value of edible mushrooms; however there is relatively little information pertaining to their antioxidant properties.

Our earlier studies on this edible mushroom *P.eous* found to have anti-inflammatory, antipyretic and analgesic activities [4, 5]. The extensive literature reveals that there are no reports on the phenol, flavonoid content of this *Pleurotus eous* mushroom apart from its pharmacological benefits. Hence we are focusing on the identification and estimation of total phenol, flavonoid content and its correlation with total anti-oxidant capacity in the present study.

MATERIALS AND METHODS

Chemicals and Reagents

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, sodium nitrite, aluminium chloride, sodium phosphate, ammonium molybdate and Quercetin were purchased from Sigma Aldrich Chemicals, Bangalore. All other chemicals, solvents and reagents were used of analytical grade and were obtained from Sisco Research Laboratories Ltd., Mumbai, India.

Collection of Mushroom fruiting bodies

The fruiting bodies of the mushroom *P.eous* were obtained from Kerala Agricultural University, Trivandrum and authenticated by Dr.Lu Lu Das, Professor, Dept of Plant Biology, College of Agriculture, Vellayani, Kerala Agricultural University, Trivandrum (Reg:No.T.5365/06:61;27/08/2009).

Methods

Preparation of the Extracts

Mushroom fruiting bodies were dried at 40-50°C for 48 hr and powdered. About 500 gm of the powdered material were extracted with petroleum ether (40-60°C) for the removal of fatty acids, waxes and sterols. The defatted material was extracted with ethyl acetate (to separate flavonoid glycosides, phenolic compounds and flavonoid) and then with 70 % methanol (for the complete removal of tannins and flavonoid compounds) for 8-10 hr using Soxhlet apparatus by hot continuous percolation method. For the preparation of aqueous extract defatted material was extracted with hot water (70-80°C) for 8-10 hours. Aliquots of extracts were collected and passed through Whatmann No.1 filter paper. Then the solvents were completely evaporated to dryness at

60ºC by vacuum distillation. The residues were designated as petroleum ether (PE), ethyl acetate (EA), methanol (MeOH) and aqueous (Aqs) extracts, respectively [6]. The extracts abbreviated as PE, EA, MeOH and Aqs are further used for their estimation of total phenol and flavonoid content and its total anti-oxidant activity evaluation.

Total Phenol Estimation

About 100 mg of the sample was dissolved in 1 ml of methanol. 50 µl of the above sample was mixed with 2.5 ml of 1:10 dilution of Folin Ciocalteu reagent followed by addition of 2 ml of Na2CO3 (7.5 % w/v). The mixture was incubated at 45°C for 15 min. Absorbance was taken at 760 nm and a standard curve was obtained using various concentrations of gallic acid. Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass) [7].

Total Flavonoid Estimation

About 100 mg of the sample was dissolved in 1 ml of water. From this 250 µl sample was taken and mixed with 1.5 ml water followed by 75 µl of $NaNO_2$ was added. Then the mixture was incubated for 6 min. 150 µl of aluminum chloride (10 %) was added to the above mixture and incubated for 5 min. The solution was mixed well and absorbance is measured at 510 nm. The calibration curve was plotted by preparing the Quercetin solutions at concentrations 100 to 1000 mg/L in DMSO [8].

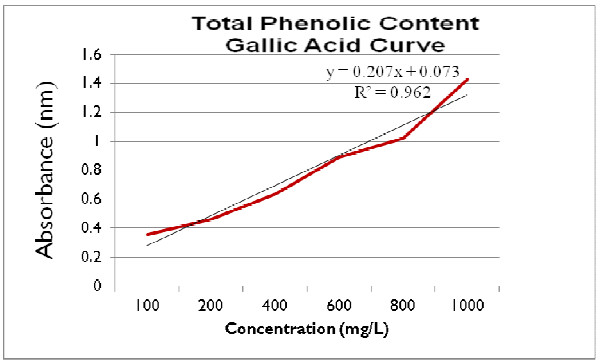
Phospho Molybdenum assay of Total Antioxidant activity

Butylated hydroxyl toluene was prepared in various concentrations (20 to 120 mg/ml) using distilled water. About 100 mg of dry mass extracts was taken in test tubes. To these 5 ml of reagent solution was added. Reagent solution was prepared by mixing sulphuric acid, sodium phosphate and ammonium molybdate in volumes of 5 ml of each respectively. All the test tubes were incubated at 30°C for 80 - 90 min. They were cooled to room temperature. The absorbance was read at 700 nm using UV - Spectrophotometer [9].

RESULTS AND DISCUSSION

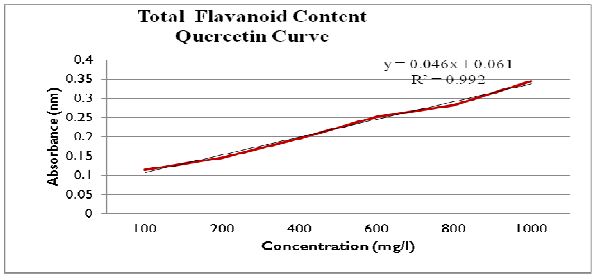
Total phenolic and flavonoid contents were estimated with the use of UV spectrophotometer. Total phenolic content was estimated by using Folin - Ciocalteu method and found to contain 146.38 mg/gm, 189.37 mg/gm, 354.59 mg/gm and 142.98 mg/gm of dry mass of PE, EA, MeOH and Aqs extracts respectively calculated from Figure 1.1. The quantitative determination of total phenolic content expressed as Gallic Acid equivalent per 100 gm dry weight of sample.

Fig.1.1 Estimation of Total Phenolic content in crude extracts of mushroom *P.eous*



Total Flavonoids content was estimated by using Quercetin as standard by aluminum chloride technique in terms of Quercetin equivalent as 93.48 mg/gm, 257.60 mg/gm, 334.785 mg/gm and 152.173 mg/gm of dry mass of PE, EA, MeOH and Aqs extracts respectively obtained from Figure 1.2.

Fig.1.2 Estimation of Total Flavonoid content in crude extracts of mushroom *P.eous*



The quantitative determination of total flavonoid content expressed as Quercetin equivalent per 100 gm dry weight of sample. Both the phenolic and flavonoid concentrations were high in methanol extract. This is attributed to its polarity. However, methanol is highly polar than ethyl acetate and pet ether. The yield of flavonoid and phenolics follows the order PE<EA<MeOH. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue). This colored complex gives the characteristic optical density in the UV measurement.

Phosphomolybdenum assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of Phospho molybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Anti oxidant capacities of extracts expressed in butylated hydroxyl toluene (BHT) equivalents. 88 mg equivalents of BHT/100 mg of extract, 56 mg of BHT equivalents/100 mg of extract, 34 mg BHT equivalents/100 mg of extracts for MeOH, EA and PE extracts respectively calculated from Figure 1.3.

Fig 1.3 Total antioxidant-phosphomolybdenum assay of crude extracts of mushroom *P.eous*

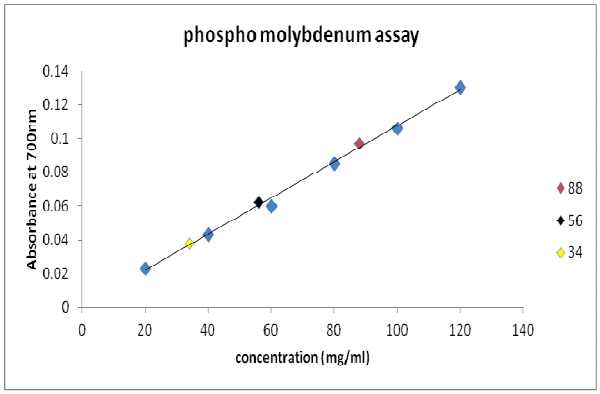
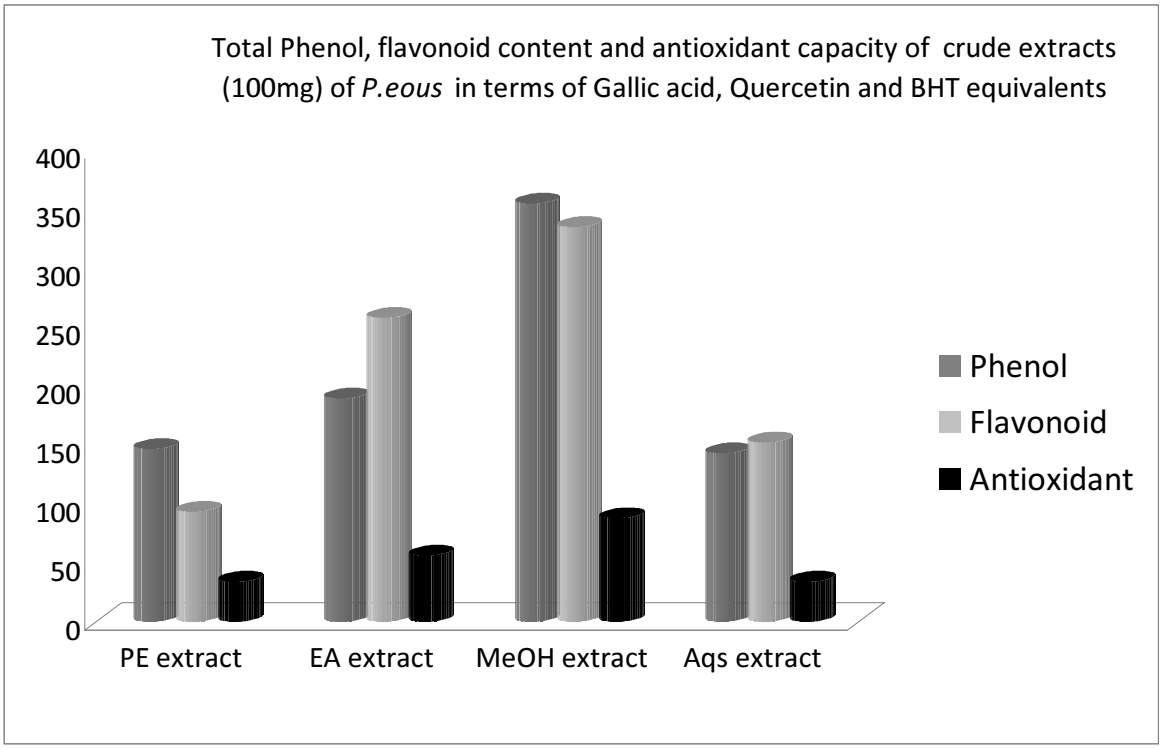


Fig.1.4: Comparison of Total phenol, flavonoid content and its antioxidant capacity of crude extracts of mushroom *P.eous*



Methanol extracts shows highest total antioxidant activity due to the presence of maximum amount of flavonoid and phenolic content which is shown in Figure 1.4. The results obtained demonstrated that methanol extract of *P.eous* had the highest total phenolic content and antioxidant activity compared to ethyl acetate, pet ether and aqueous extracts. The crude extracts had showed synergism effect which is depicted in Figure.1.4. There was a good correlation between total phenol content and antioxidant activity that support the idea of phenols as major contributor of the antioxidant power of this crude extracts.

A significant and positive correlation between total flavonoid content and phosphomolybdenum assay was observed for the crude extracts of mushroom *P.eous*. This indicated that flavonoid compounds were also the part of contributor of antioxidant activity in this mushroom.

CONCLUSION

The present study throws the light on the efficacy of methanol in the extraction of flavonoids and phenols. These findings suggest that phenolic content could be used as an indicator of antioxidant properties. The poly-phenols from the fruiting bodies can be potent antioxidants at physiological concentrations. Therefore, more fruiting bodies should be recommended to be added to the diet. Thus the mushroom *P.eous* can be used as a natural anti oxidant agent, which is proved in this work.

Acknowledgement

The authors are thankful to the VIT University management for their generous support and facilities.

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